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The invasive species Cercopagis pengoi in Lake Ontario I. Position in the food web II. Impact on mirex concentrations in the biota III. Temporal changes in mirex concentrations in salmon

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The invasive species *Cercopagis pengoi* in Lake Ontario

I. Position in the food web

II. Impact on mirex concentrations in the biota

III. Temporal changes in mirex concentrations in salmon

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Biographical Sketch

The author was born on . in Rochester, New York. Undergraduate schooling was completed at Nazareth College of Rochester in 1996 with a major in Biology and a minor in Anthropology. Upon completion of undergraduate degree, the author was employed at Columbia Analytical Services, an environmental laboratory in Rochester, New York. At Columbia Analytical, she worked as a metals analyst, analyzing samples for metals by Atomic Emission Spectrometry with Inductively Coupled Plasma, Atomic Absorption by graphite furnace, and mercury samples by a Flow Injected Mercury Analyzer. A Master of Science degree was completed in 2002 at SUNY Brockport.

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Introduction

The introduction of exotic species into ecosystems is one of the most ecologically damaging effects human activity has had on nature (Elton 1958; Ricciardi and Rasmussen 1998). The Laurentian Great Lakes have been a major acceptor of exotic species via ballast water transfers from ocean going vessels from international ports (Mills *et al.* 1993). Many of the most recent invaders are native to the Ponto-Caspian region (Mills *et al.* 1993; MacIsaac *et al.* 1999; Ricciardi and MacIsaac 2000). Such invaders include zebra and quagga mussels (*Dreissena polymorpha* and *D. bugensis*), round gobies (*Neogobius melanostomus*), and the amphipod *Echinogammarus ischnus* (MacIsaac *et al.* 1999; Ricciardi and MacIsaac 2000). The most recent invader of the Great Lakes is a pelagic, predatory zooplankter, the cladoceran *Cercopagis pengoi* (Makarewicz *et al.* 2001; MacIsaac *et al.* 1999).

Cercopagis pengoi was first observed outside of its native waters in the Baltic Sea in 1992 (Ojaveer and Lumberg 1995). Mitochondrial DNA analyses indicate that the Baltic Sea population was founded by ancestral Black Sea populations (Cristescu *et al.* 2001). In North America, *Cercopagis pengoi* has been observed in Lake Ontario, Lake Michigan, Lake Erie, St. Lawrence River, and a growing number of the Finger Lakes in Upstate New York (Makarewicz *et al.* 2001, MacIsaac, Personal Communication). Lake Ontario, the epicenter of the invasion in North America, was colonized by haplotypes characteristic of the Baltic Sea and Black Sea (Cristescu *et al.* 2001) in August of 1998 (Makarewicz *et al.* 2001, MacIsaac *et al.* 1999).

Two different morphs of *Cercopagis* were observed in Lake Ontario, *Cercopagis* (*Cercopagis*) *pengoi* and *Cercopagis* (*Apagis*) *ossiani* (Makarewicz *et al.* 2001). Mitochondrial DNA analysis indicated that these two different morphs were the same species. *C. ossiani* is the "first generation" that develops from a resting egg, while *C. pengoi* is a parthenogenetic form developing from asexual eggs (Makarewicz *et al.* 2001). Similar polymorphisms in Cladocera have been observed (e.g., *Bythotrephes, Daphnia pulex* and *D. cucullata* (I.K. Rivier, personal communication).

Cercopagis abundance in Lake Ontario is comparable to other water bodies (Makarewicz *et al.* 2001). Average maximum abundance in the nearshore of Lake Ontario reached 6,000 organisms/m^3 in 1999 (Makarewicz *et al.* 2001). Offshore abundance of *C. pengoi* remained low throughout the spring until July, when the population increased to a maximum average abundance of 1,759 individuals per cubic meter on 19 August 1999 (Makarewicz *et al.* 2001). *C. pengoi* populations then declined in the fall and were absent during the winter months (Makarewicz *et al.* 2001).

Question 1: What is the Position of *Cercopagis* in the food web?

In the Gulf of Riga, Baltic herring (*Clupea harengus*) feed on *C. pengoi* and may even prefer *C. pengoi* to the native species of zooplankton (Ojaveer and Lumberg 1995). It is assumed that *C. pengoi* feeds on nauplii, copepodites, and adult calanoid copepods, but data is lacking (Rivier 1998). In Lake Ontario, presence and absence data suggest that alewife (*Alosa pseudoharengus*) are feeding on *C. pengoi* (Bushnoe 2001). Also, experimental laboratory evidence (McPhedran 2001) suggests that *C. pengoi* consume whole rotifers and attack cladocerans, while field observations indicates that *C. pengoi* may have a predatory

impact on *Daphnia retrocurva* and *Diacyclops thomasi* in Lake Ontario (Laxson 2001). *C. pengoi* appears to be an additional seasonal link in the Lake Ontario pelagic food web during the summer months. However, further details of its position within the food web are not known.

The measurements of stable isotopic ratios of nitrogen ($\delta\ ^{15}N$) and carbon ($\delta\ ^{13}C$) have recently been employed to infer long-term aquatic food-web interactions. Heavier isotopes (^{15}N and ^{13}C) are enriched in the organism's tissues while the lighter isotopes (^{14}N and ^{12}C) are preferentially excreted (Peterson and Fry 1987). Stable isotopic ratios of nitrogen are specifically used to determine the trophic level of an organism, as there is a stepwise enrichment of 3-5‰ of ^{15}N through each trophic level of the food web, where there is only a 1-3‰ enrichment in ^{13}C between each trophic level (Minagawa and Wada 1984, Peterson and Fry 1987). Because there is little fractionation in carbon signatures between trophic levels, they are often used to determine predator-prey interactions; predators have similar carbon signatures as their prey (DeNiro and Epstein 1978). We investigated *C. pengoi's* position in the Lake Ontario pelagic food web by utilizing stable isotopes of nitrogen and carbon and by looking at alewife stomach contents.

Question 2: Will *Cercopagis* Impact Mirex Concentrations in the Biota?

Mirex (dodecachloropentacyclo[5.3.0.$0^{2,6}$.$0^{3,9}$.$0^{4,8}$]decane), an organochlorine insecticide, is a major contaminant of Lake Ontario sediments and biota (Environment Canada 1977; Armstrong and Sloan 1980). Hooker Chemical and Plastics Corporation and Armstrong Cork Company manufactured mirex from 1959 through 1976 within the Lake Ontario watershed, and these companies are responsible for the release of and subsequent

contamination of the lake with mirex (Comba *et al.*1993; Kaiser 1978). Mirex was first discovered in Lake Ontario fish in 1974 and is found throughout the food web (Kaiser 1974). In 1976, the Canadian Ontario Ministry of the Environment and the Ministry of Natural Resources concluded that all fish species tested from Lake Ontario contained mirex; however, the salmonines were the only species that exceeded the 0.10 mg/kg United States Food and Drug Administration (FDA) guideline for human consumption (TFM 1977). Subsequently, the use of mirex as a pesticide was banned in Canada in 1977 and in the United States in 1978 (TFM 1977, Kaiser 1978).

Contaminant concentrations in salmonines of Lake Ontario are a concern to fishery managers (Jackson 1997). The question becomes, what happens to mirex concentrations in top-level predators if a predatory species of zooplankton is injected into the middle of a food web? If *C. pengoi* is an additional link in the Lake Ontario pelagic food web, as preliminary data suggests, mirex concentrations should increase in the alewives and salmonids as a result of biomagnification (Rasmussen *et al.* 1990, Cabana and Rasmussen 1994). Lakes with longer food chains tend to have top predators with higher levels of lipophillic contaminants (Cabana and Rasmussen 1994; Kidd *et al.* 1995; Rasmussen *et al.* 1990; Van Hoof *et al.* 1997). We hypothesized that the greatest change in mirex concentration would become apparent first at the trophic level directly above *C. pengoi*, the alewives. We expect that if *C. pengoi* is an important food source for alewives and acts as an additional link in the food web during the summer months, concentrations of mirex should increase as the abundance of *C. pengoi* increases.

Question 3: Have the mirex concentrations in salmonines changed over the past twenty-two years

Estimates suggest that during a forty-year period, 2,700 kg of mirex entered the Lake Ontario ecosystem, of which only 550kg have been removed by transport to the St. Lawrence estuary (Comba *et al.* 1993). Like most organochlorine compounds, mirex is generally unreactive, breaking down photochemically, with the primary photolytic product being 8-monohydro mirex, or photomirex (Carlson *et al.* 1976, Mudambi and Hassett 1988), which is also unreactive and toxic (Chu *et al.* 1981).

Mirex is not readily metabolized by most organisms (Dorough and Ivie 1974, Ivie *et al.* 1974a, Ivie *et al.* 1974b) and biomagnifies in the food web, increasing in concentration with each step in the food chain (Gobas *et al.* 1993). This is a concern to Lake Ontario fish eaters, as the salmonines are inedible according to the 0.1mg/kg FDA action limit for mirex. Both sport fishing enthusiasts and fishery managers are interested in the residence time for mirex in Lake Ontario and more importantly how long will the salmonines remain contaminated.

Published information on temporal trends in contaminant levels of mirex in fish from Lake Ontario since the mid 1970s is meager (Armstrong and Sloan 1980). Trend analysis of lipophilic contaminant levels in fish has often been based solely on average concentrations. Trend analysis is of limited use in determining historical trends due to the confounding effects of fish age on weight and lipid content and therefore contaminant concentrations (Insalaco *et al.* 1982). A more effective method of analyzing temporal trends is to evaluate concentrations of the contaminants as a function of weight for each year of the trend analysis. Historical trends in contaminants can then be determined by evaluating the slope

and elevation of a regression line of concentration versus weight using analysis of covariance (ANCOVA) with weight as the covariate. SUNY Brockport graduate students have been using this approach to monitor temporal trends in mirex concentration in coho and chinook salmon *(Oncorhynchus kisutch* and *O. tschawytscha*, respectively) since 1977. Since we analyzed mirex concentrations in salmonids for the impact analysis of *Cercopagis* on Lake Ontario, we used our data to determine the temporal trends in mirex concentrations in coho and chinook salmon from 1977 to 1999.

Methods

Sample Collection

Seasonal *Cercopagis* samples were collected weekly from May through November of 2000 for abundance measurements. *Cercopagis* was collected using a double Bongo net (571μm mesh size, 50-cm diameter) following the method of Makarewicz *et al.* (2001). The contents of each net were washed into catch buckets, transferred to bottles and preserved with 10% buffered formalin. The entire sample was counted because the tendency for the spines to tangle and organisms to clump together prevented effective subsampling. Seasonal zooplankton samples were also collected weekly from May through September of 2001 for abundance measurements. Zooplankton was collected using a Wisconsin net (63μm mesh size, 50-cm diameter). The contents of the net was washed into a catch bucket, transferred to bottles and preserved with 10% buffered formalin. In the laboratory, zooplankton were identified and enumerated to the species level following methods of Gannon (1971). In general, after thorough mixing, a Hensen-Stemple pipette was used to withdraw 10-mL sub-sample aliquots, which were enumerated in a multichambered glass counting cell. The first

20 whole organisms of each species were measured. Zooplankters were measured from the anterior margin of the helmet to either the base of the tailspine (cladocerans) or the base of the caudal setae (copepods). Only whole organisms were enumerated.

Zooplankton samples for pesticide analysis were collected during the summers of 2000 and 2001. *Cercopagis pengoi, Holopedium giberum, Daphnia retrocurva, Diacyclops thomasi* and *Limnocalanus macrurus* samples were collected using a double Bongo net (571- μm mesh size, 50-cm diameter) or a Wisconsin net (63-μm mesh size, 50-cm diameter) during their seasonal population peaks. *Mysis relicta* samples were collected using an epibenthic sled (571-μm mesh size) at a depth of 100 meters. Before samples were frozen, a representative portion of the sample was visually examined for relative percent composition. *Limnocalanus*, *Diacyclops* and *Mysis* samples were generally 98% pure, while *Daphnia*, *Holopedium, Cercopagis*, and *Leptodora* samples were 75% pure. Samples were placed in solvent rinsed glass jars, kept in ice and transported back to the lab and immediately frozen until analysis.

Lake trout (*Salvelinus namaycush*), brown trout (*Salma trutta*), rainbow trout (*Oncorhynchus mykiss*), chinook (*Oncorhynchus tshawytscha*) and coho salmon (*Oncorhynchus kisutch*) were collected during their spawning run in the fall of 1999. Salmonids were collected either by electroshocking of Sandy Creek, Hamlin, New York, a tributary on the south shore of Lake Ontario, or by gill netting in Lake Ontario near the mouth of Sandy Creek. Alewives (*Alosa pseudoharengus*) were collected monthly from May through November 2000 by gillnetting. Floating gill nets were set in 6 m of water West of Sandy Creek (43 °21.347' latitude and 77° 55.077' longitude). Fish length, weight, sex and age were determined by standard procedures (Jearld 1983). Salmonines were aged

by counting scale annuli, while alewives were aged by counting annuli on the otoliths (Robert O'Gorman, personal communication; Watson 1964).

For chemical analysis of salmonines, a standard fillet consisting of the entire side from just behind the operculum to the tail, including the skin, bones of half the rib cage and one pelvic fin, but excluding the vertebrae, dorsal, pectoral, anal, and caudal fins (Armstrong and Sloan 1980), was taken from the fish, homogenized using a food processor and stored in solvent rinsed glass jars in the freezer (4°C) until pesticide analysis. Whole alewives were frozen in food storage bags. Prior to analysis, fish were thawed, guts were removed and the entire fish was homogenized using a food processor.

Diet Analysis

Immediately after collection, alewives were measured for total length and weighed, their otoliths were removed for age determination, and stomachs were flushed into vials with 10% buffered formalin. Diet analysis followed Strus and Hurley (1992). All organisms in the entire stomach were counted, except when the most abundant species numbered more than 200, in which cases a subsample was counted. Concentration of the dominant organism was adjusted to at least 100 individuals per 3ml by adding water to the stomach contents. If sub-sampling was necessary, three 3mL aliquots taken with a Hensen-Stempel pipette from each sample were examined in a glass counting dish. All prey items in the stomachs were identified to species level and counted, while the lengths of the first 20 whole organisms of each species were measured. Recognizable body parts were counted for invertebrates that were not intact. Spines were not used to enumerate *Cercopagis*, as spines tend to have longer retention times in fish stomachs than soft-bodied zooplankton parts

(Parker *et al.* 2001); instead heads were counted. The total numbers of organisms in the stomachs were estimated from the subsample by direct proportion (Mills *et al.* 1995).

Species composition was compared to ambient species composition using Ivlev's electivity index (Ivlev 1961):

$$E_i' = (r_i - p_i) / (r_i + p_i);$$

Where r_i and p_i represent the proportion of food item *i* in the diet and in the ambient water, respectively. Electivity indices vary from –1 to +1, in which a –1 indicated avoidance, 0 indicated random feeding, and a +1 indicated a preference.

Mirex Analysis

Mirex analysis followed Makarewicz *et al.* (1993), revised from Insalaco *et al.* (1982). Five grams of thawed homogenized fish sample was weighed out and mixed with 20 grams of anhydrous sodium sulfate. Excess water was removed from the zooplankton and *Mysis* samples by blotting with a kimwipe. The samples were then weighed for a wet weight determination. Zooplankton (*Daphnia*, *Holopedium*, *Limnocalanus*, *Cercopagis* and *Diacyclops*) and *Mysis* samples were placed overnight in a drying oven at 60°C and reweighed for dry weight determination, the dried sample was then mixed with 20 grams of anhydrous sodium sulfate. The sample was extracted overnight (16± 4 hrs) in a Soxhletic extractor (a minimum of 200 cycles) with 75-mL of methylene chloride/hexane (20:80 v/v) solvent mixture. A 15-mL aliquot from the salmonine extraction, a 30-mL aliquot from the alewife extraction and a 75-mL aliquot from *Mysis* and zooplankton extractions were concentrated to 1-mL under nitrogen gas, and then cleaned-up through a 5-g florisil column (at a rate of 4-mL/min) to a volume of 50-mL. This eluant was then concentrated under

nitrogen gas to a final volume of 1-mL for the salmonine and alewives, and 0.1-mL for the mysids and zooplankton. Prior to clean-up, percent extractable lipid content of salmonine and alewife was determined by evaporating a known volume of the extract and weighing the residue (Hesselberg *et al.* 1990).

Mirex and photomirex were quantified by electron capture (^{63}Ni) gas chromatography utilizing a Hewlett Packard Gas Chromatograph model 5890A with a HP7673A auto injector, a HP3396A integrator, and a wall coated open tubular fused silica capillary column (30m x 0.25mm x 0.25 μm) HP-5 (5%-Diphenyl- 95%-dimethylsiloxane) for photomirex and a (12m x 0.2mm x .33μm) Ultra-2 (dimethylpolysiloxane) for mirex. The samples were transported through the column with a 95:5% argon/methane carrier gas and the column flow was set at 0.75 ml/min. The injection port temperature was set at 250°C. The temperature program consisted of an initial temperature of 80°C, holding for 1 minute, programmed at 5°C/min to 275°C, then held for 11 minutes. A model 7673A autosampler was used to make a 50:1 split injection.

Two separate standard curves were used for low-level zooplankton mirex and photomirex determination and for higher-level salmonine and alewife mirex and photomirex determination. 10-monohydromirex was not detectable in alewives and zooplankton. Extraction blanks, replicates, and test recoveries from spiked samples were used for quality control (Appendix I-IV).

Mirex confirmation was performed on a Hewlett Packard G1800C GCD plus (Agilent Technologies, Palo Alto, Ca) with electron impact ionization. A wall coated open tubular fused silica capillary column (30m x 0.25mm x 0.25μm) HP-5MS (Agilent Technologies, Palo Alto, Ca) was directly interfaced to the GCD for the chromatographic

separation. Helium was used as the carrier gas and the column flow rate was set at 1.0 ml/min. The injection port temperature was set at 280°C. The temperature program consisted of an initial temperature of 70°C, holding for 2 minutes, programmed at 20°C/min to 260°C, then held for 15 minutes. A model 7673 autosampler was used to make a splitless (1 minute) injection. The instrument was operated in selective ion monitoring mode (SIM) for the detection of the following ions corresponding to mirex: m/e's 203, 237, 238 and 272.

Stable Isotope Analysis

All samples were analyzed for stable isotopes of carbon and nitrogen at Cornell-Boyce Thompson Stable Isotope Laboratory (CoBSIL), where a mass spectrometer (Finnigan Delta Plus) interfaced to a Carlo Erba elemental analyzer dedicated for detecting stable isotope ratios of carbon and nitrogen in solids was employed. Homogenized samples were freeze-dried at -40°C and ground to a fine powder with a mortar and pestle. Sample were weighed out (0.5mg) on a Sartorius microbalance MC5 (readable to 1μg) and placed into tin capsules (3.5 x 5mm) prior to the analysis.

The capsules with sample were combusted at 1000 °C to produce gasses of CO_2 (for carbon isotope ratios) and N_2 (for nitrogen isotope ratios), and transported to the Faraday cup detector by an ultra-high purity helium carrier gas. The isotopic ratio was then calculated: $\delta X = (({}^{15}N/{}^{14}N \text{ of sample})/({}^{15}N/{}^{14}N \text{ of standard})-1) \times 1000$. This calculation was also used for isotopic ratios of carbon (${}^{13}C/{}^{12}C$). The results are in parts per thousand deviations from the standard. The nitrogen standard was the nitrogen in atmospheric N_2 and the carbon standard is the carbon in PeeDee limestone (Peterson and Fry 1987).

Statistical Analysis

All statistical analyses were done using SPSS 10.0 (SPSS Inc.): Analysis of variance (ANOVA) tests were used to test for differences in nitrogen and carbon signatures between species. *Holopedium* and *Leptodora* results for carbon and nitrogen signatures were not included in these tests, as these samples had low variances compared to other species and violated the equal variance assumption of ANOVA. ANOVA was also used to test for significant differences in mirex concentrations among zooplankton species or groups. When replication was low (n= 2 to 6) and the null hypothesis was not rejected, the statistical power of the ANOVA was determined (Cohen 1969) to expose the probability of rejecting the null hypothesis when there is an effect present (Peterman 1990).

Logarithmic transformations were required to correct for heteroscedastic variances in alewife mirex and photomirex concentrations to meet the assumptions of ANOVA. A two factor ANOVA was used to test for differences in alewife mirex concentration between month, age class and the interaction of age class and month. Two factor ANOVAs were also used to test for differences in photomirex concentration, weight, and percent lipid between month, age class, and the interaction of age class and month as well. Linear regressions tested for relationships between the dependent variable of alewife mirex concentration and the independent variables of fish weight, percent lipid, and fish length.

ANOVA was used to test for temporal trends in average annual mirex concentrations of salmonines independent of weight, and Analysis of Covariance (ANCOVA) was used to test for temporal trends in mirex concentrations with salmonine weight as the covariate and weight x sampling year as an interaction term. A significant interaction term indicated that the slope of the mirex concentration-salmon weight regression line was dependent on the sampling year. Slopes of

each regression line were compared using a pair-wise t-test of all possible pairs, in which the significance levels were corrected using Bonferroni layering (Darlington 1990). Regression line elevations were also analyzed for significant differences using a Tukey HSD test of the least square means (LSMEANS) for each sampling year. The LSMEANS are the means for the salmon mirex concentration after they have been adjusted for the covariate of weight.

Results

Position of Cercopagis in the Lake Ontario Food Web

Mirex Analysis

There was an increase in mirex concentration of at least one order of magnitude with each step (ie. from zooplankton to forage fish and from forage fish to salmonines) in the Lake Ontario food web (Figure 1). The salmonines were clearly the top predators, but the mirex concentrations in the lake trout were at least four times those of the other species of salmonines (Table 1 and Figure 2). Organisms low on the food web had higher concentration factors. For example, there was a 100-fold increase in mirex concentration from *Daphnia* or *Cercopagis* to the alewife, while there was only a 10-fold increase in mirex concentration from the alewife to the salmonine. *Mysis*, being a large predator and associated with the benthos, had mirex concentrations 50 times those of the other species of zooplankton.

Mirex concentration in *Limnocalanus macrurus* was 0.0008mg/kg, which is much higher than the average mirex concentrations for *Cercopagis pengoi*, *Leptodora kindtii,*

Holopedium gibberum, *Dalphnia retrocurva* and the single *Diacyclops thomasi* sample (Table 1). Because only one replicate of *Limnocalanus macrurus* and *Diacyclops thomasi* sample were available for analysis, they were grouped together into a group called "copepods" for statistical analysis. ANOVA found no significant difference ($F=2.55$, $df=5,14$, $p= 0.077$) in the mirex concentrations of *Cercopagis pengoi, Leptodora kindtii, Holopedium gibberum*, *Daphnia retrocurva*, "copepods" and *Mysis relicta.* However, the power of this analysis was 0.37, indicating that there was a 63% chance of not rejecting the null hypothesis when there was an effect present. The average effect size was 0.0046 mg/kg. More replication may have revealed a significant difference between zooplankton groups.

Isotope Analysis

Usually, there is a 3-5‰ difference in nitrogen signatures between adjacent trophic levels (Minagawa and Wada 1984, Peterson and Fry 1987). Salmonines are clearly the top predators in the Lake Ontario pelagic food web with nitrogen signatures ranging from 13.91 to 18.53‰ (Figure 3). Alewives, the major forage fish in Lake Ontario, are a trophic level below the salmonines with nitrogen signatures ranging from 11.73 to 13.79‰. Based on nitrogen signatures, *Cercopagis pengoi*, *Daphnia retrocurva, Leptodora kindtii*, and *Holopedium gibberum* appear to all be at the same trophic level, with nitrogen signatures ranging from 7.15 to 9.14‰. The rotifers appear to be a trophic level below *Cercopagis pengoi, Daphnia retrocurva, Leptodora kindtii*, and *Holopedium gibberum* with lower nitrogen signatures ranging from 5.14 to 6.27‰. *Limnocalanus macrurus* and *Mysis relicta* have enriched nitrogen signatures that are more similar to alewives than zooplankton, with

signatures ranging from 12.99 to 14.2‰ and from 9.84 to 12.47‰, respectively. ANOVA results confirm these observations ($F=174.5$, $df=10,73$, $p<0.001$, Table 1).

Mirex concentrations in representative species from the Lake Ontario pelagic food web were significantly correlated ($F=191$,$df=1,61$, $r^2=0.76$, $p<0.0001$) with the nitrogen signatures that describe their trophic position (Fig 4).

The carbon signatures of biota characteristic of the Lake Ontario pelagic food web ranged from –31.76 to –21.81‰ (Table 1 and Figure 3). Rotifers, *Holopedium gibberum, Leptodora kindtii, Cercopagis pengoi*, alewives, rainbow trout, brown trout, coho and chinook salmon all had similar carbon signatures, ranging from -25.96 to -21.81‰. *Limnocalanus macrurus*, *Daphnia retrocurva*, *Mysis relicta* and lake trout had isotopically lighter carbon signatures ranging from -31.76 to -26.55‰. ANOVA followed by a Tukey HSD revealed that the carbon signatures of lake trout, *Daphnia retrocurva*, *Limnocalanus macrurus*, and *Mysis relicta* were significantly different (ANOVA, $F=34.85$, $df=10,73$, $p<0.001$) from those of the rotifers, *Cercopagis pengoi*, alewives, and the other species of salmonines (Table 1).

Alewife Stomach Analysis

Alewife stomach contents generally matched seasonal changes in the most abundant prey species available in the water (Tables 2 and 3). For example, *Diacyclops thomasi* was the most abundant species of zooplankton in the ambient water in May and made up 35% of the alewife diet. However, calanoid copepods represented only 4.5% of the zooplankton community, but represented 59% of the diet of alewives.

In June, July and August, *Diacyclops thomasi* and *Bosmina longirostris* were the most abundant prey species and had the highest percent abundance in the alewife stomachs. *Cercopagis pengoi* was not observed in plankton tows in July, but made up 41% of the alewife diet during that month. *Bosmina longirostris* and *Daphnia retrocurva* were most abundant in the water samples in September and October; both were the major prey items in the alewife stomach in September, making up 43 and 42% of the stomach contents, respectively. In October, *Bosmina longirostris* composed 76% of the alewife diet and *Pontoporeia* made up 23%.

Mean monthly values for Ivlev's electivity index show fairly consistent negative (avoidance) values for *Bosmina longirostris, Daphnia retrocurva, and Cercopagis pengoi* in May, June, July, August, September and October (Table 4). However, *Bosmina longirostris* is the major food item for alewives in August and *Cercopagis pengoi* was preferred (+0.60) in July. In August, no species were positively selected for; however, *Bosmina longirostris* had the least negative Ivlev's index of –0.34. There was no consistently positive (preference) value for any one species throughout the sampling period. For example in May, Ivlev's index indicated that *Mysis relicta* was the preferred species (+0.17) while in June *Holopedium gibberum* and *Diacyclops thomasi* were preferred (+0.20 and +0.64, respectively). In July, a greater variety of organisms were observed in alewife stomachs. Preferred organisms included *Holopedium gibberum* (+0.20), *Polyphemus pediculus* (+0.20), *Cercopagis pengoi* (+0.60), *Leptodora kindtii* (+0.20), and *Mysis relicta* (+0.40). During the months of September *Pontoporia* (+1), calanoid copepods (+0.25) and *Polyphemus pediculus* (+0.50) were selected. By October, selection focused on *Pontoporia* (+1) and *Canpthocampus* (+0.20).

Trends in Alewife Mirex Concentration with Changing Cercopagis *Abundance*

At the offshore sampling location, a maximum of 11.7 *Cercopagis*/m^3 were observed in the months of May, June and mid-July 2000. At the end of July, the population increased dramatically; in one week the population increased from 35 individuals/m^3 on 27 July to 434 individuals/m^3 on 3 August. The population continued to increase until the maximum abundance of 680 *Cercopagis*/m^3 on 19 August. After this peak, there was a sharp decline in the population to 275 *Cercopagis*/m^3 on 24 August. The population fluctuated around 200 organisms/m^3 in September and slowly decreased through November to only one organism/m^3 on 13 November (Figure 5).

Average monthly mirex concentrations in the 1996 (age four) alewife year class were significantly higher (two-factor ANOVA, $F= 22.28$, $df=1,4$, $p<0.001$) than average monthly mirex concentrations in the 1998 (age two) alewife year class in 2000 (Figure 6 and AppendixVI Table A). No increase was observed in the monthly mirex concentration in alewives after the peak abundance of *Cercopagis*. Actually, the alewife mirex concentration during the month of September was significantly lower than those in May or June (two-factor ANOVA, $F=3.16$, $df=1,4$, $p=0.022$ Appendix VI Table A). Average monthly photomirex concentrations in the 1996 (age four) alewife year class were also significantly higher (two-factor ANOVA, $F=4.19$, $df=1,4$, $p=0.046$) than average monthly mirex concentrations in the 1998 (age two) alewife year class in 2000, but no changes in monthly alewife photomirex concentrations were observed (Appendix VI Table B). However, photomirex values should be viewed with some caution, as fish extracts were not analyzed

for 8-photomirex by gas chromatograph until eight to nine months after extraction (Appendix V).

A two factor ANOVA also found no significant differences in monthly alewife weight and no differences in the interaction of age and month on fish weight, but the weight of the 1996 alewife year class was significantly higher ($F=50.72$, $df=1,4$, $p<0.001$) than that of the 1998 year classes (Table 5 and Appendix VI Table C). Similarly, there was no significant difference in monthly alewife percent lipid, but strangely the 1998 year class had a significantly higher (two-factor ANOVA, $F=5.86$, $df=1,4$, $p=0.019$) lipid content than the 1996 year class (Table 5 and Appendix VI Table D). We expected to see a significant increase in percent lipid throughout the summer as the fish prepare for winter, but this was not observed. There were no significant correlations between alewife mirex concentration and total length ($R^2=0.07$, $p=0.14$, $df=1,32$), weight ($R^2=0.01$, $p=0.54$, $df=1,32$) or percent lipid ($R^2=0.02$, $p=0.44$, $df=1,32$).

Temporal changes in mirex concentrations in salmon

Significant differences (ANOVA, $F=7.32$, $df=5,115$, $p<0.001$) were observed in mirex concentrations in salmon over the twenty-two year period (Tables 6). Tukey HSD tests revealed that average mirex concentrations in salmon collected in 1999 were lower ($p< 0.05$) than in all other years of collection (Table 6). No other consistent temporal trend was obvious. Average mirex concentration decreased from 0.22 to 0.19 mg/kg in the 1977 to 1986 period, increased to 0.24mg/kg by 1992, and after 1992, the average mirex concentration decreased to 0.08 mg/kg in 1999. Comparison of percent lipid content from 1986 to 1999 found no significant changes (ANOVA, $F=0.099$, $df=3,70$, $p=0.96$) (Table 6).

Within any given sampling year, mirex concentration was a function of weight (Fig .6, also Insalaco *et al.* 1982). If mirex availability to salmon were the same over time and if no ecological changes took place, a similar relationship of concentration versus weight should exist over the twenty-two year study period. That is, average mirex concentration would be a function of average weight of fish analyzed. This was true to some extent, as the highest average mirex concentration observed was for a year that had the second highest average fish weight (1992). However, the year with the highest average fish weight, 1999, had the lowest average mirex concentration (Table 6). Clearly, other factors were influencing mirex concentration and fish weight should be taken into account in trend analysis rather than just employing simple averages of toxic concentration over time.

To account for the differences in average fish weight between each collection year, the temporal trends in mirex concentration in salmon were evaluated by considering the slope of the regression line of mirex concentration versus fish weight for each collection year using ANCOVA with weight as the covariate (Figure 7). Pair-wise t-test comparisons of the slopes of the ANCOVA regression lines for each collection year indicated that the slope of the 1999 regression line was significantly different ($df=1,5$, $p=0.014$, in Table 7) from the slopes of the regression lines from all previous years (1977, 1982, 1986 and 1992) except 1996 ($df=1,5$, $p=0.966$). Slopes for the 1977, 1982, 1986, 1992 and 1996 ANCOVA regression lines were not significantly different ($df=1,5$, $p \geq 0.077$ in Table 7). The slopes for the 1996 and 1999 regression lines were flatter (Figure 7), indicating that the mirex concentrations in the larger fish were decreasing. In 1999, mirex concentrations in salmon weighing 1.0 to 12 kg were below the United States FDA guideline for human consumption of 0.1mg/kg for mirex (TFM 1977), whereas only salmon weighing less than two kilograms were below this guideline in previous years (Figure 7). Interestingly, by 1999, the

regression line of mirex concentration versus fish weight was not significantly different from zero ($F=1.22$, $df=1,17$, $R^2=0.07$, $p= 0.21$), in contrast to all previous years in this study (Figure 7).

Utilizing the least square means (LSMEANS) of the weight adjusted mirex concentrations from the ANCOVA analysis, we compared the difference in the elevations of each regression line (Table 6). The weight adjusted mean mirex concentrations decreased from 0.273 in 1977 to 0.067 in 1999. A Tukey test (Table 8) revealed that the elevation of the 1977 regression line was significantly higher than that of all other years ($p\leq0.005$) and that the elevation of the 1999 regression line was significantly lower than that of all other years ($p<0.001$). Elevations of LSMEANS of 1982, 1986 and 1992 were not significantly different ($p\geq0.129$), while the elevation for the 1996 regression line was significantly lower ($p\leq0.008$) than those of 1977, 1982, and 1986, but not significantly different from ($p=0.306$) 1992. The elevations of the weight versus mirex concentration regression lines seems to have been decreasing over time, which suggests that the mirex concentrations per kilogram of fish has been decreasing over time. There have been significant decreases in regression line elevations on two different occasions. The first decline occurred between 1977 and 1982, after the use of mirex as a pesticide was banned in the United States and Canada. The second major decline occurred between 1996 and 1999; the cause of this decline is not well understood.

Discussion

The New Food Web - Stomach Analysis of Alewives

The similarity in the $\delta^{13}C$ between alewives and *Cercopagis* suggested that alewives were feeding on *Cercopagis*, but stomach analysis was necessary to determine how important this species of zooplankton was to the alewife diet. The species that were selected for in the alewife diet during at least one month included: *Cercopagis, Leptodora, Daphnia, Holopedium, Bosmina, Diacyclops*, calanoid copepods, *Mysis*, and *Pontoporia*, which agrees with the finding of Mills *et al.* (1992). Alewives generally have a tendency to eat the most abundant prey item, generally *Diacyclops* and *Bosmina,* and when zooplankton were abundant, they select the larger prey items (Mills *et al.* 1992). This was apparent in the 2001 analysis of the 1998 (age 3) alewife year class stomachs, as *Mysis, Pontoporia, Polyphemus*, and *Holopedium* were the major species selected. *Cercopagis* is both a large and abundant prey item, but it was only selected for during July, and even the abundance of *Cercopagis* in alewife stomachs was highly variable for this month. This suggests that alewives do not feed as heavily on *Cercopagis* as might be expected based on data from the Baltic Sea (Ojaveer and Lumberg 1995).

These results at first appear to be contradictory to the year 2000 alewife stomach analysis conducted at Cornell University, which concluded that *Cercopagis* spines were present in all alewives examined that were larger than 80mm (Bushnoe 2001). However, the population size of *Cercopagis* in the ambient water in 2000 was much greater than the 2001 population. Furthermore, the enumeration of spines does not accurately represent the number taken on a given day because of digestion retention (Parker *et al.* 2001), and without

accurate measurements of the entire diet for alewives in 2000 it is difficult to infer preference relationships. Alternatively, alewives may have developed a learned response to avoid *Cercopagis*, as they may have suffered the previous year from large boluses of spines in their stomachs. Parker *et al.* (2001) observed large boluses of *Bythotrephes* spines in the stomachs and throats of rainbow smelt in Lake Erie and speculated that these fish may not be feeding because they feel satiated.

The New Food Web – Isotope Data

Ratios of heavy to light isotopes are expressed as δ values called "signatures" expressed as (‰) part per thousand deviation from the standard. In comparing two samples, a more positive δ value indicates heavy isotope enrichment, or increased amounts of the heavier isotope. Conversely, a lower δ value indicates the heavier isotope is depleted, or amounts of the heavier isotope are decreased and amounts of the lighter isotope are increased (Peterson and Fry 1987).

Isotope ratios in aquatic organisms are dependent on biogeochemical cycling, reactions, and the concentration and isotope signatures in the dissolved inorganic carbon and nitrogen pools used by primary producers. The carbon isotope signatures of dissolved inorganic carbon is determined by the relative concentrations and forms of dissolved inorganic carbon and the processes influencing the chemical and isotope equilibrium between components of the lake carbonate system (Leggett 1998). The general rule is that the heavy isotopes concentrate in the molecule where the bond strengths are the greatest (Peterson and Fry 1987). Therefore, bicarbonate contains more ^{13}C and is enriched

compared to CO_2. The concentration of $CO_{2(aq)}$ in the lake is dependent on pH, temperature, alkalinity, the remineralization of $CO_{2(aq)}$, and the amount of photosysnthesis taking place.

The hypolimnion of thermally stratified lakes tends to have organisms with depleted carbon signatures compared to epilimnetic organisms as a result of more ^{12}C available in the dissolved inorganic carbon pool of the hypolimnion (Leggett 1998). There are several reasons why the hypolimnion contains more ^{12}C:

1. CO_2 has more depleted carbon signatures than bicarbonate and is more soluble in colder water.
2. Primary producers are mainly found in the epilimnion, where they utilize the lighter isotope of carbon from the dissolved inorganic carbon pool first. If the level of photosynthesis is large relative to the dissolved inorganic carbon pool being drawn from, eventually the dissolved inorganic carbon pool will become enriched in ^{13}C and the primary producers will draw from this thereby enriching their carbon signatures. Because no mixing take place between the epilimnion and hypolimnion, the carbon signatures in the dissolved inorganic carbon pool of the hypolimnion remain depleted, as well as the organisms drawing from this pool.
3. The remineralization of CO_2 adds $CO_{2\,(aq)}$ to the dissolved inorganic carbon pool as a product of respiration. Respired CO_2 has depleted carbon signatures. Therefore, if respiration provides a significant contribution to the overall dissolved inorganic carbon pool, the carbon signatures of this pool will be lower than the equilibrium value.

Carbon signatures of the biota characteristic of the Lake Ontario pelagic food web were significantly different, indicating the different physical location and possible food

source of organisms within the pelagic food web (Figure 3). Carbon signatures in rotifers, *Cercopagis*, *Leptodora*, *Holopedium*, alewives and brown trout, rainbow trout and coho and chinook salmon are all within the same range (-25.96 to –21.81‰), suggesting predator-prey interactions among these organisms. Carbon signatures of lake trout, *Daphnia*, *Mysis* and *Limnocalanus* are significantly lower than those of the rotifers, *Holopedium*, *Cercopagis, Leptodora,* alewives and other species of salmonines. The slightly depleted carbon signatures of *Limnocalanus* and *Mysis* may be a result of their physical location in the water column while the lower value for *Daphnia* may be a function of sampling time. Organisms, such as *Mysis* and *Limnocalanus*, located in hypolimnetic waters of a stratified lake can be depleted in ^{13}C compared to epilimnetic organisms as a result of either the enrichment of the dissolved inorganic carbon pool in the epilimnion from large amounts of photosynthesis taking place or benthic algae were uptaking respired CO_2 that can be abundant in the deep water of stratified lakes (Rau 1978, 1980).

A similar mechanism may be the cause of the reduction in ^{13}C in Daphnia observed in this study. *Daphnia* samples for isotope analysis were taken in October after thermal stratification had broken down. Thermal mixing delivers hypolimnetic ^{12}C, which is the utilized more quickly by algae (Schelske and Hodell 1991), to organisms throughout the water column, thereby causing carbon signatures to be depleted (Leggett 1998).

Alewives are the main forage base for salmonines in Lake Ontario (Brandt 1986, Elrod and O'Gorman 1991) and are reflected in the carbon signatures of rainbow trout, brown trout and coho and chinook salmon. Carbon signatures in lake trout were more depleted, which suggests a diet other than strictly alewife. While adult lake trout feed on alewife and smelt, juvenile lake trout (age two and yearling) are known to prefer benthic

organisms depleted in ^{13}C such as slimy sculpin, johnny darters, isopods, amphipods and *Mysis* (Elrod 1983, Elrod and O'Gorman 1991). This is consistent with the findings of Kiriluk *et al.* (1995) who discovered that the carbon signatures of immature lake trout reflected a diet dominated by slimy sculpin and adult lake trout had slightly enriched carbon signatures reflecting a diet in alewife and smelt.

Stable isotopic ratios of nitrogen indicate that the rotifers are at the base of the trophic web, with nitrogen signatures significantly lower than those of *Cercopagis* and *Daphnia*. Laboratory predation experiments of *Cercopagis* on native species of zooplankton have found that *Cercopagis* preferred the rotifer *Asplanchna* to the cladocerans: *Ceriodaphnia*, *Daphnia* or *Moina* (McPhedran 2001). Based on nitrogen signatures, *Cercopagis* and *Leptodora* appear to be within the same trophic level as *Daphnia* and *Holopedium* even though they are predatory species of zooplankton. Nitrogen signatures clearly demonstrate nutrients moving from the zooplankton (*Cercopagis, Daphnia, Holopedium* and *Leptodora*) to the alewives and ultimately the salmonines (Figure. 3).

Stable isotope results indicate that the ratio of ^{15}N to ^{14}N in *Daphnia* and *Holopedium* are similar to those of *Cercopagis* and *Leptodora*. This was surprising. We would expect this ratio to increase, as *Cercopagis* and *Leptodora* are predators. For every step in the food chain, there is an increase in the ratio of ^{15}N to ^{14}N by 3-5‰ (Minagawa and Wada 1984). However, there are other factors that affect the variability in the isotope ratio, such as the organism's location in the water column during thermally stratified periods and time (Leggett 1998). Ratios of ^{15}N to ^{14}N also change over time, as organisms preferentially retain the heavier isotope (Minagawa and Wada 1984).

Why are two predaceous species such as *Leptodora* and *Cercopagis* not clearly separated in stable isotope signatures of nitrogen from the herbivorous species of zooplankton, *Daphnia* and *Holopedium*? We do not have a good explanation. *Holopedium, Leptodora, Daphnia* and *Cercopagis* were collected at different times during the summer. To gather large and pure samples of a species, organisms were collected when peak abundance occurred. Thus *Cercopagis* was collected in early August and September, *Holopedium* and *Leptodora* in late August and early September and *Daphnia* in October. The time span between species collections may be long enough to observe changes in ratios of ^{15}N to ^{14}N due to preferential uptake. If the ratios did change, zooplankton samples would be depleted in ^{15}N as the summer progressed and ^{15}N in the dissolved inorganic nitrogen pool was used up (Leggett 1998). However, no such decline in nitrogen signatures from *Cercopagis*, collected in August, to *Daphnia*, collected in October were observed.

Another potential explanation for the lack of separation between the nitrogen signatures of the herbivorous and predatory zooplankton is their vertical position in the water column. Under pre-*Cercopagis* conditions in Lake Ontario, *Daphnia, Holopedium, Leptodora*, and *Cercopagis* inhabit the epilimnion of stratified water bodies (Makarewicz *et al.* 2001). Laxson (2001) has suggested that the predation pressure of *Cercopagis* may be causing *Daphnia, Holopedium* and other prey species to remain in the metalimnion of Lake Ontario for prolonged periods of time. During the summer, ^{15}N availability is greater outside of the epilimnion, where it is not being depleted as rapidly (Leggett 1998). If *Daphnia* and *Holopedium* migrate to the metalimnion for prolonged periods, their nitrogen signatures may be enriched. There is evidence that *Daphnia* did vertically migrate into the metalimnion of Lake Ontario during the summer of 2001(Meyers 2001 Unpublished data), however the

migration of *Daphnia* into the metalimnion has been observed in the Great Lakes prior to the invasion of *Cercopagis* (Wells 1960, McNaught and Hasler 1966).

The nitrogen signatures of *Mysis* and *Limnocalanus* suggest that these organisms are within the same trophic level as the alewife. Based on our stomach analysis (Tables 2,3 and 4) and the research of others (Mills *et al.* 1992, 1995, Urban and Brandt 1993, Iancu 1989), we know that alewives prey upon *Limnocalanus* and *Mysis*, suggesting other factors are affecting their nitrogen signatures. The enriched nitrogen signatures in *Limnocalanus* and *Mysis* may also be a result of their physical position in the water column. Benthic primary consumers, such as *Mysis* and *Pontoporeia*, feed on decomposing phytoplankton and detritus that have enriched nitrogen signatures, resulting in the enrichment of nitrogen signatures in benthic and hypolimnetic organisms. This elevated signature is not indicative of an elevated trophic position for these species (Vander Zanden and Rasmussen 1999). In addition, *Mysis* may have elevated nitrogen signatures as a result of denitrification and ammonification processes that occur in anoxic regions of stratified lakes. Both processes fractionate nitrogen isotopes considerably; causing an enriched pool of ^{15}N that is available for primary producers (Wada and Hattori 1978, Macko and Estep 1985, Owens 1987). However, it is unlikely that denitrification and ammonification are responsible for the enrichment of nitrogen in *Mysis*, as anoxia has not been observed in the deep waters of Lake Ontario.

By conducting stomach analysis on alewives, analyzing stable isotopes of nitrogen and carbon and the mirex concentrations in keystone species from each trophic level in Lake Ontario, it is apparent that *Cercopagis* does not represent a new trophic level or step in the Lake Ontario pelagic food web. Based on our analysis of stable isotopes of nitrogen and the laboratory predation experiments conducted at the University of Windsor (McPhedran 2001),

it appears that *Cercopagis* preys upon rotifers in Lake Ontario. Stable isotopic ratios of nitrogen failed to separate *Cercopagis* from other large-bodied herbivorous species of zooplankton (*Daphnia* and *Holopedium*). Furthermore, there was no significant difference between the mirex concentrations of *Cercopagis, Leptodora, Daphnia,* copepods and *Holopedium.* Stable isotopic ratios of carbon and nitrogen suggest that nutrients are flowing from *Cercopagis* to alewives, which was confirmed by alewife stomach analysis, but *Cercopagis* was only present in the alewife stomach for a relatively short period of time. Stable isotope results conclude that the ratio of ^{15}N to ^{14}N in *Daphnia* and *Holopedium* are similar to that of *Cercopagis* and *Leptodora*.

Impact of *Cercopagis* on Mirex Concentrations in the Biota

Does the introduction of *Cercopagis pengoi* impact the mirex concentrations in the fish, primarily alewife? Even though seasonal abundance of *Cercopagis* peaked in August and average mirex concentration in *Cercopagis* populations were two times greater than those of the herbivorous zooplankton *Holopedium gibberum*, there were no significant seasonal increases in average mirex concentrations in age two 1998 and age four 1996 alewife year classes (Figure 6). We conclude that the insertion of this new species into the Lake Ontario food web has not affected mirex levels in alewives.

In fact, mirex concentrations in September alewives (age four) were significantly lower than those in May and June (Figure 6). This result is presumably an artifact of the analysis procedure used, as we did not remove the eggs of the age four fish prior to analysis. May and June alewives carried eggs containing elevated levels of lipids and mirex, which upon homogenization elevates the level of mirex reported. Seasonally, there was no change

in the monthly mirex concentrations in the (age two) 1998 alewife year class (Figure 6), as eggs were removed from all fish prior to pesticide extractions. Egg release is an elimination pathway for mirex in the (age four) 1996 alewife year class.

There may be several reasons why we did not observe an increase in alewife mirex concentrations over the summer. Perhaps it is because *Cercopagis* reached high abundances for only a short period of time during the year. High abundances of *Cercopagis* only occurred in Lake Ontario during August (Figure 5), which may not be long enough to have a significant impact on alewife mirex concentrations. That is, the load of mirex from ingesting *Cercopagis* may actually be low despite the higher abundance and elevated concentrations in *Cercopagis* during the summer. Modeling may provide insight into the impact that *Cercopagis* would have on mirex concentrations in alewives if it were abundant in the lake for longer periods of time.

A second hypothesis is that alewives may be avoiding *Cercopagis* as a food source. There is evidence to support this hypothesis. Our stomach analysis revealed that alewives generally avoided *Cercopagis* except during July when abundance was moderate and alewives selected for *Cercopagis* (Ivlev's index= +0.60).

We suggest that no increases in alewife mirex concentrations were observed over the summer because *Cercopagis* does not represent an "extra step" of significance in the Lake Ontario pelagic food web, eventhough energy and materials (i.e., mirex) passes through them from one level of the trophic web to another.

As expected, the biomagnification of mirex was significantly correlated to the trophic level of an organism as described by nitrogen signatures (Figure 4, also Kiriluk *et al.* 1995). These findings agree with other studies (Rasmussen *et al.* 1990, Cabana and

Rasmussen 1994, Kidd *et al.* 1995, Kiriluk *et al.* 1995, VanHoof *et al.* 1997) that have examined this relationship between contaminant biomagnification and trophic level, according to $\delta^{15}N$. Such research discovered that trophic level, predator-prey interactions, and food web length are responsible for much of the variability in contaminant concentrations in piscivorous fish between lakes (Rasmussen *et al.* 1990, Cabana *et al.* 1994). Based on these results, contaminant concentrations are expected to increase in the fish if an additional link in the food web was made by the insertion of a predatory species of zooplankton. However, significant changes in mirex concentrations in the Lake Ontario food web as a result of the invasion of *Cercopagis* to the Lake Ontario pelagic food web were not observed.

Temporal changes in mirex concentrations in salmon

Any changes in food web mirex concentrations would eventually become apparent in salmonine body burdens. The sportfishing, seafood, and commercial fishing industries in New York State generate a total of $11.5 billion worth of economic activity annually (NY Sea Grant 2001). In Lake Ontario, the salmonine sport fishery (lake trout, brown trout, rainbow trout, coho salmon, and chinook salmon) is pre-eminent. An estimated 188,210 anglers fished Lake Ontario for a total of 2.5 million days in 1996 (Connelly *et al.* 1999). Over $170 million dollars were spent on sport fishing trips to New York's Great Lakes waters in 1996 (Connelly *et al.* 1999, NY Sea Grant 1998). However, because of contamination by mirex and other chlorinated contaminants, a consumption limit and health advisory exists on fish from Lake Ontario. Women of childbearing age, infants and children under the age of 15 are advised not to eat any salmonines, while others are limited to one serving per month of fish under the length of 20 inches; larger salmonines and all

sizes of chinook salmon should not be consumed (NYSDOH 2001). For mirex, the FDA action limit is 0.1mg/kg; that is, the FDA will take legal action to remove all products from the market that have mirex concentrations at or above 0.1 mg/kg. For the past 20 years in Lake Ontario, coho and chinnok salmon greater than 2 kg in weight have exceeded the FDA action limit for mirex.

Despite these consumption advisories, there is evidence (Bush *et al.* 1983, Madden and Makarewicz 1997) that mirex is entering into the human food chain. For example, women who ate salmon from Lake Ontario had increased levels of mirex and photomirex in their breast milk compared to women who ate panfish (i.e. perch, sunfish and bass) or did not eat any fish from Lake Ontario (Madden and Makarewicz 1997). Since lactation provides the only elimination pathway for mirex and other lipophilic contaminants, these toxins can be transferred to infants during breast-feeding (Gallenberg and Vodicnik 1989). Also, mirex levels in lactating women geographically near Lake Ontario are slightly higher, but not significantly higher, than those of women further away from the lake (Bush *et al.* 1983).

Halfon (1981) suggested that it would take 200 to 600 years before mirex-contaminated sediments were completely covered by mirex free sediments. Scrudato and DelPrete (1982) agree with this estimate based on the sedimentation rates of Kemp and Harper (1976) and sediment concentrations near the Oswego River and Niagara River anomalies (Holdrinet *et al.* 1978, Scrudato and DelPrete 1982). Mirex is predicted to have such a long residence time in the biota of Lake Ontario because it is one of the most stable compounds ever discharged into the lake (Metcalf *et al.* 1973) and because the nearshore bottom sediments redistribute into the water column, providing a continual source of mirex to the pelagic biota (Scrudato and DelPrete 1982). Also, biota associated with the contaminated sediment and important in the food web, such as *Mysis* and *Pontoporeia*, could continue to deliver mirex into the food web for many years, perhaps hundreds of years (Evans

1982, Marzolf 1965, Whittle and Fitzsimons 1983). However, results presented here suggest that either this is not happening or it is not a significant problem. Within twenty-four years of mirex being banned, mirex LSMEANS concentrations (Table 8) in salmon fillets have decreased significantly. In 1999, most salmonines below the weight of 12 kg were below the 0.1mg/kg FDA action level for mirex, whereas seventeen to twenty years ago only juvenile salmon were below that level and from thirteen to three years ago only fish smaller than 2kg had concentrations below the action level (Figure 7). Similarly, there is a general consensus that PCB concentrations in Lake Ontario lake trout decreased greatly between the 1970s and the 1990s (Borgmann and Whittle 1992, Huestis *et al.* 1996, DeVault *et al.* 1996).

Interestingly, the model of Flint and Stevens (1989) that considered mirex loss through the food web, subsequent sedimentation, sediment burial, removal of mirex by the harvest of fish and loss from outlets of the St. Lawrence River predicted an elimination of mirex from the Lake Ontario water column by 2010. This model appears to be more consistent with our findings than the Halfon (1981) model. The major difference between the Halfon (1981) model and the Flint and Stevens (1989) model is that Flint and Stevens assumed that there were no new sources of mirex in Lake Ontario, whereas Halfon (1981) assumed that contaminated sediments from the Oswego and Niagara rivers would be resuspended into the water column and deliver mirex to the food web for many years.

The question becomes what is the cause of the significant reduction in the LSMEAN mirex concentration in salmonine fillets? The proximal cause has to be a reduction within the levels of the trophic web, while ultimately, there is most likely a loss of mirex from the water column. Hydrophobic contaminants, such as mirex, are readily removed from the water by adsorption to particulates, which are taken up by phytoplankton (e.g., Harding and Phillips 1978, de la Cruz and

Naqvi 1973). It follows that a reduction in lake productivity, with an accompanying reduction in the amount of matter being produced at each trophic level, should lead to a reduction in the mirex concentrations in biota because theoretically, less organic matter and lipids containing mirex are available to organisms per unit time. In Lake Ontario, the Phosphorus Abatement Program is responsible for successfully reducing the loadings of phosphorus to Lake Ontario. As a result of this reduction, ambient levels of phosphorus have decreased, causing phytoplankton abundances to decrease and water clarity to increase (Millard *et al.* 2000). Thus an overall decrease in lake productivity may be responsible for the declining trends of mirex contamination in salmon. As the biomass of organisms low on the food chain decrease (Millard *et al.* 2000), there should be less mirex available to organisms at the next trophic level, resulting in a decrease in mirex at each trophic level.

The introduction of the zebra mussel (*Dreissena polymorpha*) and the quagga mussel (*Dreissena bugensis*) in Lake Ontario in the late 1980s may have also contributed to water clarity and removal of mirex from the pelagic food web. Zebra mussels have incredible filtering capacities, and therefore could potentially accumulate high levels of contamination from the water or particle-bound contaminants such as mirex. Ultimately, mirex could be removed from the water column (water, particulate matter, and phytoplankton) to the benthic region by the accumulation of mirex in zebra mussel tissue. We have estimated the total mass of mirex bound up in zebra mussel tissue in Lake Ontario for 1991-1992 (25.7kg) and 1995 (2.8kg) based on mean zebra mussel abundance data from Haynes *et al.* (1999). These estimations are liberal, as the entire surface area of the lake was used to determine entire lake abundance for zebra mussels. The 25.7kg of mirex in zebra mussels in 1991-1992 is comparable to the 28kg of mirex reported in fish in 1981 (Comba *et al.* 1993). However, it appears that there has been a dramatic decline in the number of zebra

mussels since the early 1990s and the 2.8kg of mirex in zebra mussel tissue in 1995 is trivial compared to the amount of mirex removed from the system by sedimentation (a total of 2000kg). The estimated amount loss to sedimentation would include that found in zebra mussels.

Indeed there is evidence of a decrease in mirex concentrations in various portions of the food web since the late 1970s exists. Mirex in alewife, a major food item of salmonines in Lake Ontario (Brandt 1986, Jackson 1997), has decreased considerably from 1976 to 2000 (Table 9). Major reductions have also been noted in yellow perch and zooplankton (Table 9). The average reductions in mirex concentrations are more than tenfold at each trophic level from 1977 to 2000 suggesting that there is less mirex available for biomagnification.

Models have demonstrated that a forage base with younger less contaminated alewife, or even less contaminated fish in general, will result in a decrease in the pesticide concentrations in top predators (Jackson 1996,1997, Rand and Stewart 1998a, 1998b, Stow and Carpenter 1994). A declining forage base of alewife in Lake Ontario (O'Gorman 1997) could also result in reduced mirex concentrations in salmon. With a smaller food ration, the salmon will not gain as much lipid as they did in previous years when alewife populations were higher, and smaller lipid reserves result in a lower capacity to retain hydrophobic contaminants (Mackay 1979, 1982, Bentzen *et al.* 1996). However, because we found that lipid content in the salmon has remained constant over the twenty-two year period, this is probably not the mechanism for mirex reductions in salmon.

Modeling efforts (Jackson 1996,1997, Rand and Stewart 1998a, 1998b, Stow and Carpenter 1994) have also suggested another pathway of reduction in salmonine mirex concentration, termed "growth dilution". These models predict contaminant concentrations in salmonines based on fish growth, amount of contaminants in the prey, amount of contaminants egested or excreted, respiration rates and specific dynamic action (metabolic costs). In these simulations, piscivore

pesticide concentration can be reduced if the intake and excretion of pesticides remains constant, but the growth of the fish increases presumably due to an increased growth rate or standing stock of forage fish (Jackson 1996,). However, since the alewife forage base in Lake Ontario was actually decreasing from 1977 to 1992 (O'Gorman 1997), it is unlikely that "growth dilution" is the cause of mirex reduction in salmon.

A more probable cause of mirex reduction in salmon is the recent reduction in alewife size. Salmon are size selective predators that attack larger alewives first. These large alewives are old, have been exposed to mirex longer, and have higher levels of contamination (Jackson 1996, 1997). It is possible that there are fewer large, highly contaminated alewives left in Lake Ontario because alewife abundance has been low in the recent years and the salmon have been readily removing these large fish over time. Temporal studies of salmon diet suggest that the number of alewives consumed has increased more than three fold from 1980 to 1993, but the average size and the mean weight of alewife in salmon stomachs have decreased at least 50% during that period (Rand and Stewart 1998a). A reduction in the size in alewives in salmon stomachs suggests that alewife sizes in general have decreased over time (presumably due to the size selective predation behavior of the salmon) and recently the younger, less contaminated alewives make up a major portion of the salmon diet. We have found that alewife mirex concentrations have been declining over the years (Table 9), which may be due in part to the fact that alewife abundance is low in the lake and alewives are not living as long and picking up as much mirex before salmon prey upon them.

Ultimately, reductions in the mirex contamination of the food web may be due to a decrease in the mass of mirex in the water column. Several potential pathways exist by which mirex mass in the water column may potentially be reduced. These include photodegradation, volatilization into

the atmosphere, sinking of organic particles containing mirex to the sediments, sport harvesting (i.e., fishing), and loss through the outlet of Lake Ontario at the St. Lawrence River.

Mirex within the water column can be chemically broken down into degradation products through different dechlorination processes (Carlson *et al.* 1976). For example, when mirex in the water is exposed to ultraviolet light (Kaiser 1978, Mudambi *et al.* 1992), photomirex or 8-monohydromirex is a degradation product. High ratios of P/M (photomirex/mirex) in organisms would indicate that mirex is being broken down into photomirex in the water and biomagnified through the food web (Flint *et al.* 1988). Over the past twenty-two years, the relatively constant P/M ratios in salmon tissue (Table 6) suggest that the decreases in mirex concentrations in salmon are not a result of photodegradation.

Unlike the relatively high amounts of PCBs lost by degassing from Lake Michigan (Arimoto 1989), the volatilization of mirex into the atmosphere is minimal (Hoff *et al.*1992). Although mirex has a low volatility from water and a high solubility in biological tissue, mirex can volatilize from a lake and be carried by the wind to land systems (Arimoto 1989). However, atmospheric transport is unlikely to effectively reduce concentrations in Lake Ontario due to a low Henry Coefficient, H= $7x10^{-4}$ (Yin and Hassett 1986). Furthermore, summer epilimnetic temperatures have not changed, so there is no basis for an increase in rate of volatilization of mirex from Lake Ontario. Major losses of mirex to the atmosphere are not likely (Hoff *et al.* 1992).

Besides volatilization and photodegradation, other potential pathways of mirex loss from the water column include transport by organisms into the terrestrial or stream habitats (Low 1983, Lewis and Makarewicz 1988), sinkage of mirex-laden organisms to the sediments and loss through the St. Lawrence River (Flint and Stevens 1989). Although demonstrated, the movement of mirex by salmonine migration into stream habitats and by aquatic insects feeding on salmonine carcasses

is minimal (Low 1983, Lewis and Makarewicz 1988). The observed reductions in mirex in salmonines appear to be fairly accurately predicted by the simulations of Flint and Stevens (1989), which suggest reductions and the elimination of mirex from the system by 2010. The apparent accuracy of this model provides insight on pathways of elimination from the Lake Ontario water column. In this model, losses of mirex through sedimentation and through the St. Lawrence River are the major pathways of loss, not through fish harvesting.

Conclusion

The invasion of the predatory species of zooplankton, *Cercopagis pengoi*, into Lake Ontario does not represent an "extra step" of significance in the pelagic food web, however it is clearly a link. The integration of results from stable isotopic analysis of nitrogen and carbon and the measurement of mirex concentrations in keystone species from each trophic level as well as alewife stomach analysis conclude that although nutrients flow from *Cercopagis* to alewives, it represents only a small portion of the alewife diet. As a result, no increases were observed in the monthly mirex concentrations in alewives.

Mirex concentrations in pelagic biota appear to have been decreasing over the years, most likely due to the removal of mirex from the water column through sedimentation processes and the removal through the St. Lawrence River. The ultimate effect has been a decrease in salmonine mirex concentration, which in 1999 has reached significantly lower concentrations, placing most 12kg fish below the FDA action limit for mirex. This study has demonstrated that the introduction of a predatory species of zooplankton did not disrupt the removal of mirex from the food web, or at least it is not apparent yet.

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Figure 1. Average mirex concentrations (mg/kg wet weight) in the Lake Ontario Food Web from organsims collected in 1999, 2000, and 2001

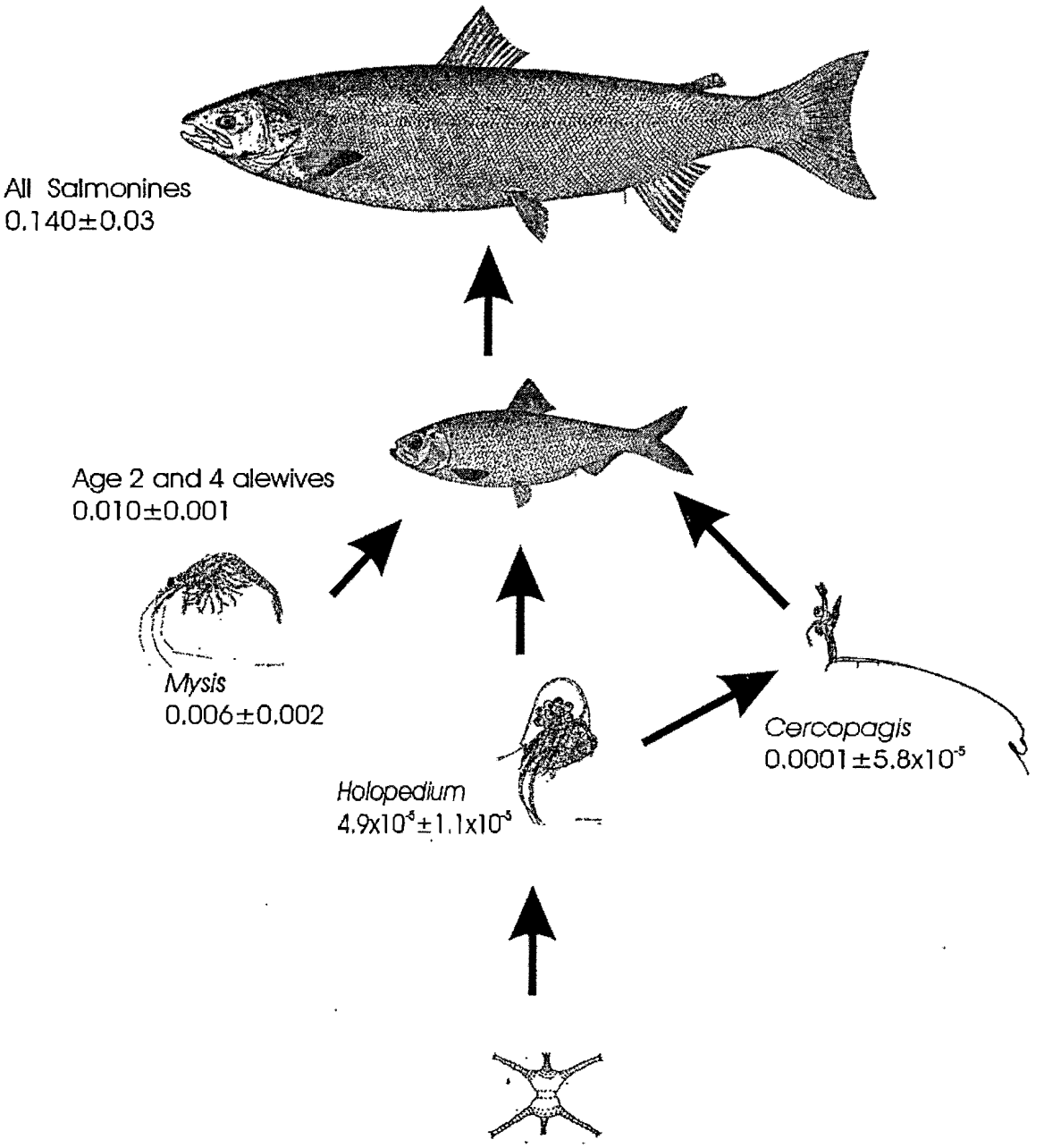


Figure 2. Mirex concentrations in representatives from the Lake Ontario pelagic food web collected in 1999, 2000, 2001. Values are means +/- standard errors.
(Insert in caption: mirex concentrations in alewives and zooplankton at a smaller scale)

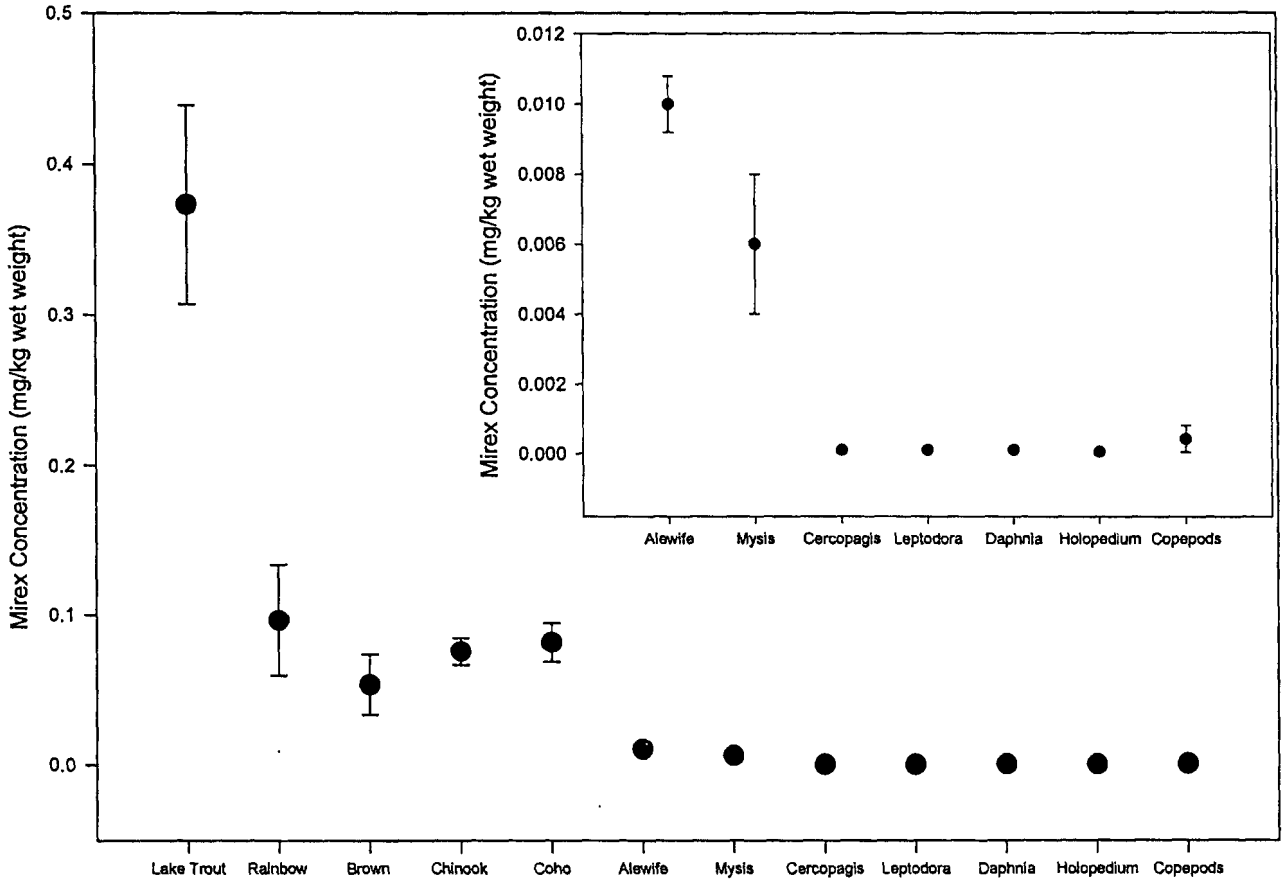


Figure 3. Lake Ontario food web as described by stable isotopes of nitrogen (delta N) and carbon (delta C) in organisms collected in 1999, 2000, and 2001.

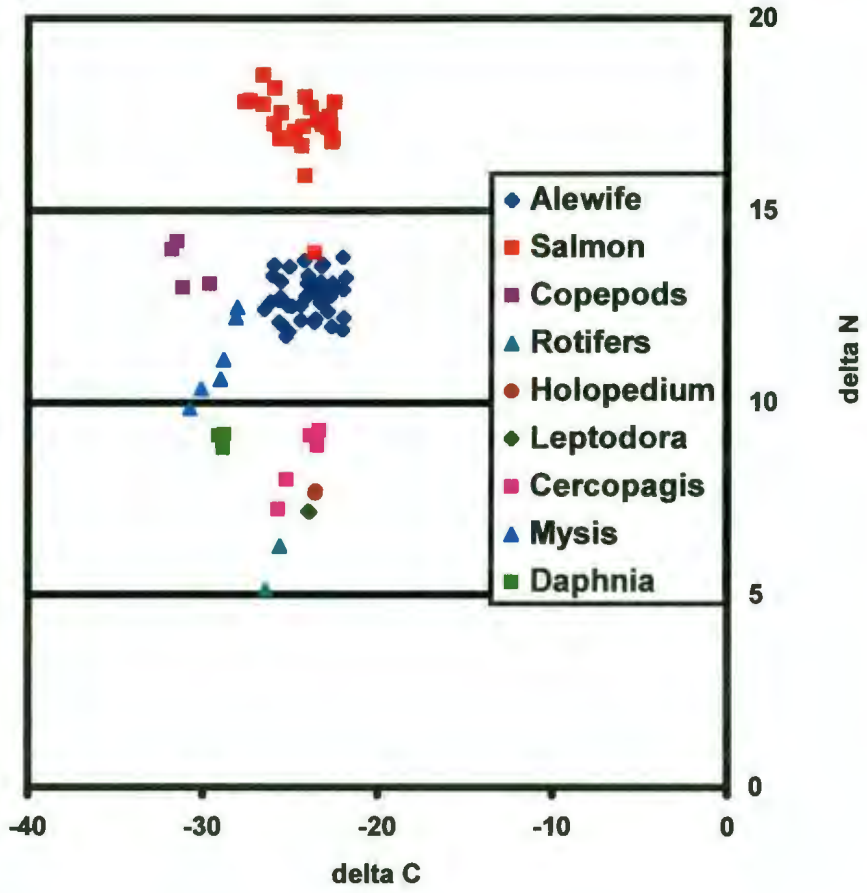


Figure 4. Relationship between mirex concentration and nitrogen isotopic signatures for biota in the Lake Ontario pelagic food web, 2000-2001.

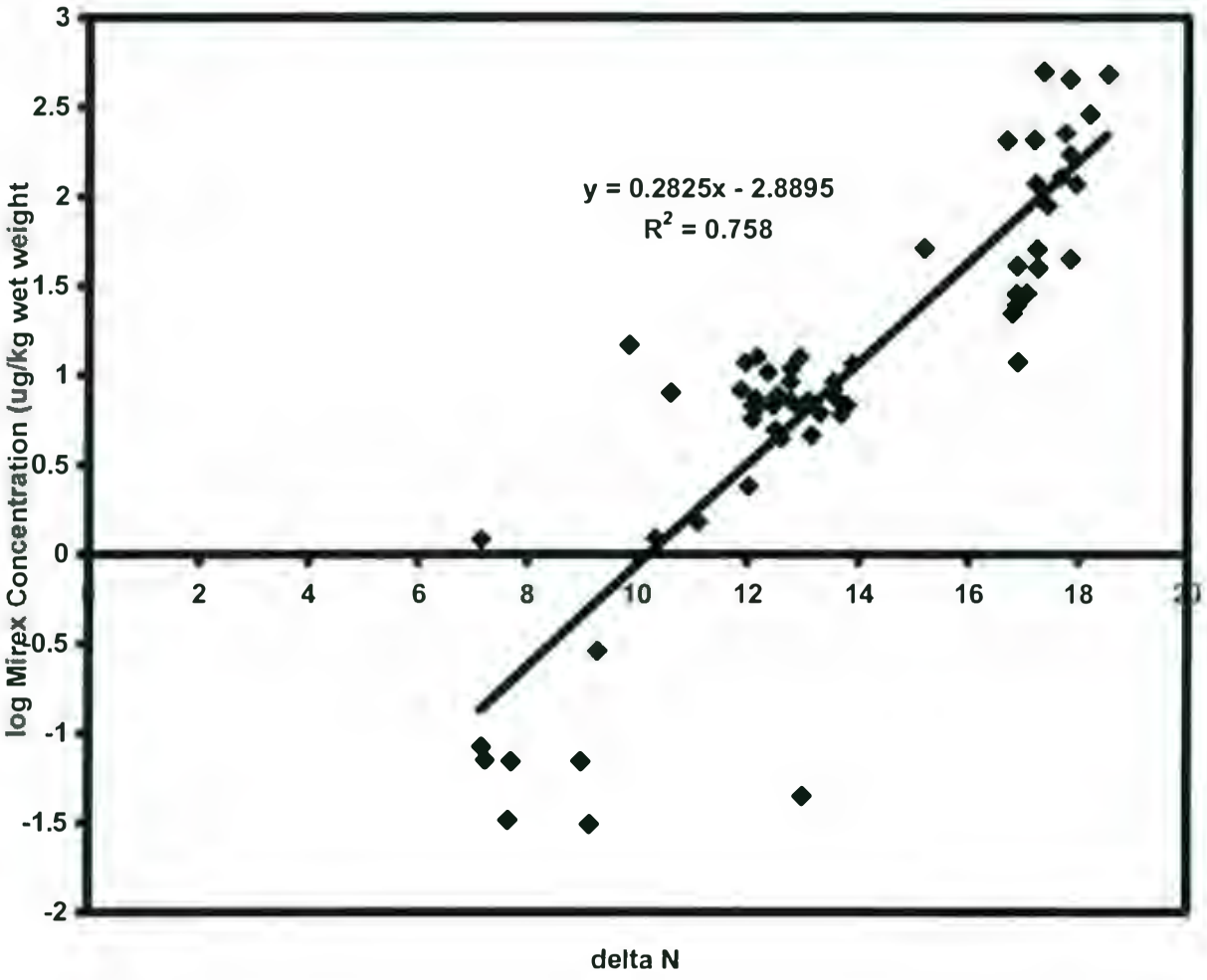


Figure 5. Offshore Abundance of *Cercopagis pengoi* in Lake Ontario, 2000. Values are average abundance +/- the standard error.

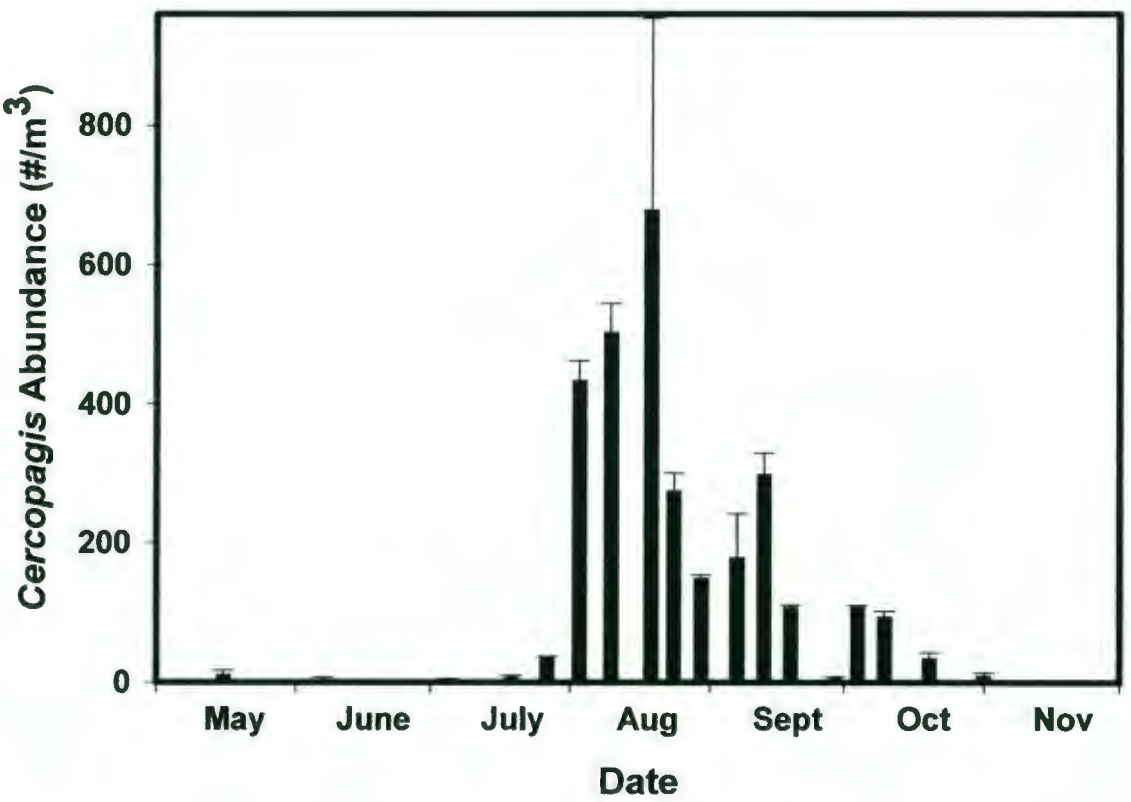


Figure 6. Mirex Concentration in (age two) 1998 and (age four) 1996 alewife year classes with Cercopagis abundance. Values are average concentrations +/- the standard error.

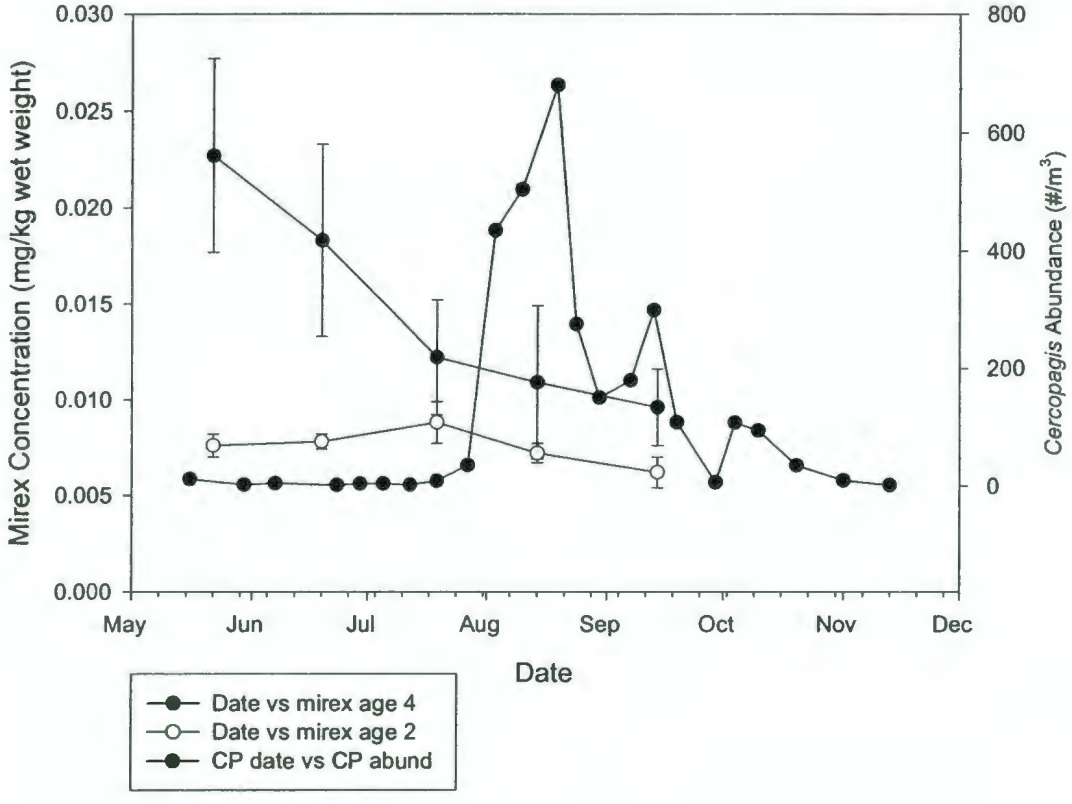


Figure 7. Relationship between mirex concentration and weight in Chinook and Coho salmon over a 22-year period. Lines represent simple regression lines.

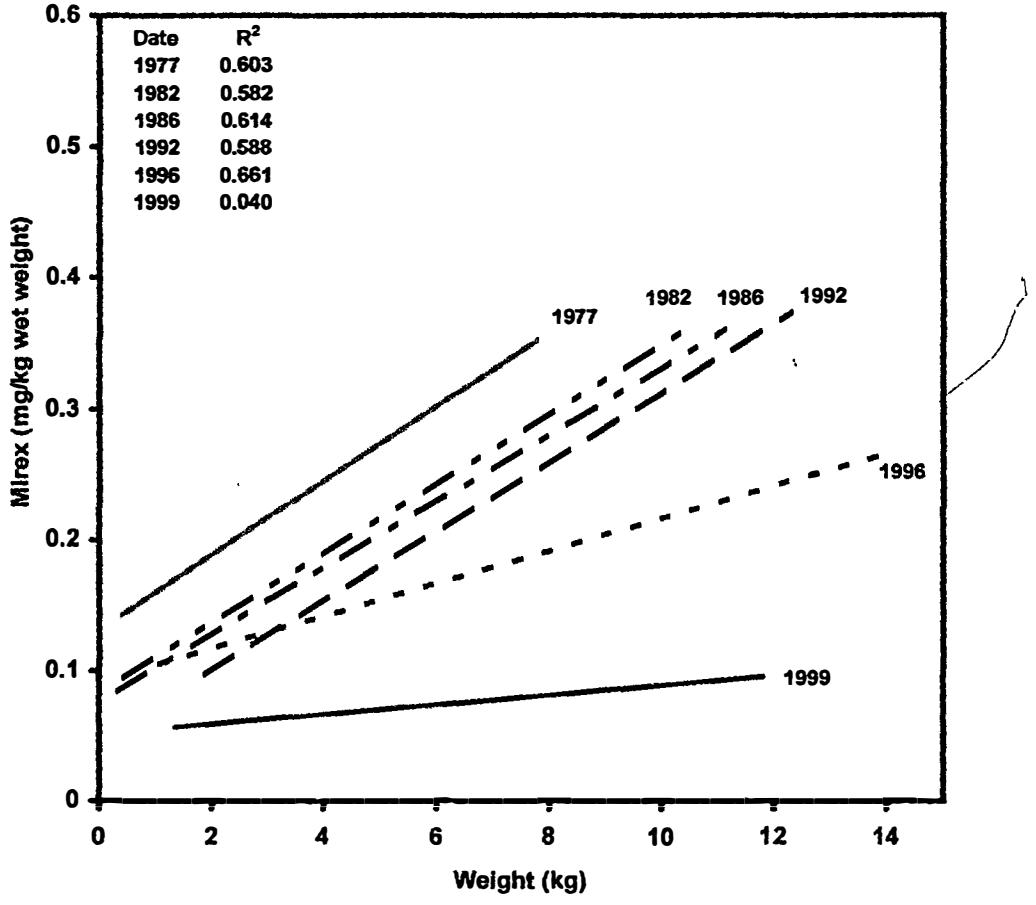


Table 1. Total length, total weight, percent lipid (fish only), isotope signatures (‰), and pesticide concentrations (mg/kg wet weight) of Lake Ontario biota. Values are means ± standard error. ND = No Data. Values followed by the same letter for a given measure are not significantly different (ANOVA, $p>0.05$). Note: * denotes samples were not used in statistical analysis because extremely low variances violated the assumptions of ANOVA.

Sample type	(Pesticide) N	Total length (cm)	Total weight (g)	%Lipid	(isotope) N	$\delta^{13}C$	$\delta^{15}N$	Mirex	Photomirex
Salvelinus nyamuch	8	68.5±0.98	457.2±50.9	22.4±2.46	5	-26.81±0.30 a	18.04±0.14 a	0.373±0.066	0.062±0.014
Salmo trutta	8	54.2±3.46	288.3±38.5	7.85±1.32	5	-25.36±0.23 ab	17.12±0.13 ab	0.053±0.020	0.016±0.002
Oncorhynchus mykiss	6	59.3±4.25	365.5±69.2	13.7±1.31	5	-24.39±0.29 bc	16.11±0.59 b	0.096±0.037	0.035±0.014
Oncorhynchus tshawytscha	13	87.9±1.24	892.0±38.3	2.90±1.22	5	-23.35±0.32 bc	17.33±0.23 ab	0.075±0.009	0.030±0.007
Oncorhynchus kisutch	6	64.3±3.96	361.0±67.2	5.77±3.11	5	-22.83±0.12 c	17.38±0.12 ab	0.081±0.013	0.031±0.009
Alosa pseudoharengus	84	14.4±1.56	40.96±4.47	21.5±2.69	39	-24.02±3.90 bc	12.75±0.09 c	0.010±0.001	0.007±0.001
Mysis relicta	6				6	-29.11±0.44 d	11.10±0.43 d	0.006±0.005	0.002±0.0004
Cercopagis pengoi	4				7	-24.04±0.37 bc	8.66±0.29 e	$0.0001\pm1.0\times10^{-4}$	$0.0001\pm6.7\times10^{-5}$
Leptodora kindtii	2				2	-23.90±0.05 *	7.16±0.01 *	$0.0001\pm2.9\times10^{-5}$	$0.0001\pm8.9\times10^{-5}$
Limnocalanus macrurus	1				4	-30.99±0.48 d	13.57±0.31 c	0.0008	0.0004
Holopedium gibberum	3				2	-23.52±0.02 *	7.67±0.030 *	$4.9\times10^{-5}\pm1.1\times10^{-5}$	$0.0002\pm5.5\times10^{-5}$
Daphnia retrocurva	3				4	-28.85±0.07 ad	9.05±0.08 e	$0.0001\pm2.6\times10^{-5}$	$0.0001\pm3.7\times10^{-5}$
Diacyclops thomasi	1				0	ND	ND	4.4×10^{-5}	0.0019
Rotifera	0				2	-25.99±0.42 ab	5.70±0.57 f	ND	ND

Table 2. Average number of major food items in stomachs of (age 3) 1998 alewife year class from May through October 2001, Lake Ontario. Values are the mean ± standard error.

Taxa	May	June	July	August	September	October
Fish examined (n)	6	4	5	5	4	5
Mean length (mm)	142± 1.8	132± 3.3	142± 2.8	134±1.6	126±1.8	140± 1.2
Mean weight (g)	20.6± 0.8	15.8± 2.0	19.6± 1.2	18.3± 0.7	18.8± 1.0	25.2± 0.6
Bosmina longirostris	0	4.6± 3.2	6.0± 2.4	2357.7± 698.3	308.7±225.5	269.9± 141.8
Daphnia retrocurva	0	2.1± 2.4	0.6± 0.4	0.5± 0.5	300.1± 281.5	0
Holopedium gibberum	0	1.1± 1.3	0.8± 0.8	0	38.3± 38.3	0
Ceriodaphnia spp.	0	0	0	0	27.8± 25.6	0
Polyphamus pediculus	0	0	0.2± 0.2	0	9.5±7.6	0
Cercopagis pengoi	0	1.3± 1.0	11.0± 10.5	0.9± 0.9	2.8± 2.0	1.1± 1.1
Leptodora kindtii	0	0	1.6± 1.6	0	0.8±0.8	0
Diacyclops thomasi	15.2± 3.4	2067.9± 776	3.4± 1.2	2917.2±2506.0	12.2±11.3	2.3± 1.5
Calanoida	25.5± 8.2	0	0	0	1.0± 1.0	0
Campthocampus spp.	0	0	2.0± 0.8	0	0	0.9± 0.9
Mysis relicta	2.8± 2.8	0	0.6± 0.4	0	0	0
Pontoporeia spp.	0	0	0	0	18.3± 4.9	80.6± 45.4

Table 3. Percent abundance of major food items in the ambient water (W) and in the stomachs (S) of (age 3) 1998 alewife year class from May through October 2001, Lake Ontario.

Taxa	May		June		July		August		September		October	
	W	S	W	S	W	S	W	S	W	S	W	S
Bosmina longirostris	4.9	0	10.2	0.2	82	22.4	39.0	44.7	29.0	42.9	38.0	76.0
Daphnia retrocurva	3.7	0	11.5	0.1	4.8	2.2	4.7	0.01	46	41.7	23.0	0
Holopedium gibberum	0	0	0	0.05	0	3.0	0	0	22	5.3	28.0	0
Ceriodaphnia spp.	0	0	35.0	0	0	0	0	0	1.2	3.8	4.0	0
Polyphemus pediculus	0	0	0	0	0	0.7	0	0	0	1.3	0	0
Cercopagis pengoi	0.01	0	0.03	0.06	0	41.0	0.06	0.02	0.04	0.4	5.0	0.3
Leptodora kindtii	0	0	0	0	0	6.0	0	0	0.32	0.1	*1.0*	*0*
Diacyclops thomasi	91.0	34.9	35.0	99.6	11	12.7	56.0	55.3	0.34	1.7	0.4	0.66
Calanoida	4.5	58.6	7.0	0	1.7	0	0	0	0	0.14	1.0	0
Campthocampus spp.	0	0	0.6	0	0	7.5	0	0	0	0	0	0.26
Mysis relicta	0	6.5	0	0	0	2.2	0	0	0	0	0	0
Pontoporeia spp.	0	0	0	0	0	0	0	0	0	2.5	0	22.7

Table 4. Mean Values for Ivlev's electivity index for (age 3) 1998 alewife year class from May through October 2001, Lake Ontario. Values are averages.

Taxa	May	June	July	August	September	October
Bosmina longirostris	-1.00	-0.92	-0.98	-0.34	-0.93	-0.74
Daphnia retrocurva	-1.00	-0.96	-0.96	-0.99	-0.96	-1.00
Holopedium gibberum	0.00	+0.20	+0.20	0.00	-0.99	-1.00
Ceriodaphnia spp.	0.00	0.00	0.00	0.00	-0.88	-1.00
Polyphemus pediculus	0.00	0.00	+0.20	0.00	+0.50	0.00
Cercopagis pengoi	-1.00	-0.26	+0.60	-0.84	-0.67	-0.99
Leptodora kindtii	0.00	0.00	+0.20	-1.00	-0.98	-1.00
Diacyclops thomasi	-0.97	+0.64	-0.92	-0.63	-0.82	-0.78
Calanoida	-0.38	-1.00	-1.00	0.00	+0.25	-1.00
Campthocampus spp.	0.00	-1.00	-0.95	0.00	0.00	+0.20
Mysis relicta	+0.17	0.00	+0.40	0.00	0.00	0.00
Pontoporeia spp.	0.00	0 .00	0.00	0.00	+1.00	+1.00

Table 5. Weight, total length, percent lipid, and pesticide concentrations (mg/kg wet weight) of (age 2) 1998 and (age 4) 1996 alewife year classes collected from Lake Ontario, 2000. Values are the means ± the standard errors.

Age 2

Date	N	Weight (g)	Length (mm)	%Lipid	Mirex (mg/kg)	8-Photomirex (mg/kg)
5/22/00	4	13.9±1.4	129±3.7	5±3.1	0.0076±0.0006	0.002±0.0008
6/19/00	4	17.6±3.1	138±7.9	9±4.0	0.0077±0.0004	0.004±0.0020
7/19/00	5	18.7±1.9	131±3.9	6±1.9	0.0088±0.001	0.010±0.0003
8/14/00	4	18.0±1.5	129±2.5	5±2.2	0.0072±0.0005	0.006±0.0014
9/14/00	17	18.6±1.0	132±3.0	10±2.4	0.0062±0.0008	0.006±0.0006

Age 4

Date	N	Weight (g)	Length (mm)	%Lipid	Mirex (mg/kg)	8-Photomirex (mg/kg)
5/22/00	4	27.6±2.6	164±5.0	6±3.1	0.0227±0.0005	0.017±0.0089
6/19/00	4	25.4±4.3	159±5.6	2±0.4	0.0183±0.0047	0.009±0.0010
7/19/00	5	29.1±0.7	157±2.5	1±0.4	0.0122±0.0028	0.005±0.0006
8/14/00	5	23.8±1.8	152±4.4	4±3.1	0.0110±0.0038	0.006±0.0017
9/14/00	5	26.4±1.7	159±2.2	10±7.3	0.0096±0.0025	0.004±0.0004

Table 6. Descriptive statistical data for salmon fillets collected from 1977 to 1999 and ANOVA results. Mirex concentrations are in mg/kg-wet weight. Percent lipid data is not available for 1977 and 1982 sampling years. LSMEANS are the weight adjusted mirex concentrations for each sampling year from the ANCOVA, and ratios of 8-photomirex to mirex (P/M) are presented. NA denotes data that is not available.

							ANOVA Results	
n	24	24	24	12	19	19		
Year	1977	1982	1986	1992	1996	1999	F	p
Mean mirex (mg/kg)	0.22	0.18	0.19	0.24	0.16	0.08	7.325	<0.001
Std. error	0.02	0.02	0.02	0.04	0.02	0.01		
Range	(0.07-0.41)	(0.03-0.35)	(0.02-0.41)	(0.09-0.48)	(0.06-0.29)	(0.021-0.26)		
Tukey test p	0.000	0.003	0.001	0.000	0.043	*		
Mean % lipid	NA	NA	3.36	3.88	3.62	3.81	0.9601	0.099
Std. Error			0.427	0.555	0.326	1.276		
Range			(0.35-9.05)	(1.9-7.9)	(1.4-6.37)	(0.52-17.28)		
Mean weight (kg)	3.20	3.69	4.46	7.12	5.62	7.23		
Std. Error	0.47	0.52	0.66	1.11	1.12	0.67		
Mean length (cm)	62.19	64.15	70.13	82.56	69.10	80.00		
Std. Error	3.41	3.15	3.67	5.32	5.23	2.96		
LSMEANS	0.273	0.215	0.203	0.178	0.153	0.067		
P/M	0.3-0.4	0.6	0.42	0.43	0.38	0.37		

Table 7. ANCOVA table and slopes of the regression lines for respective years for the relationship between fish weight and mirex concentration (ANCOVA). Also, p values from a pair-wise comparison of the slopes of the regression line for each year with a Bonferroni Layering correction. Note: p Values < 0.05 indicate a significant difference.

ANCOVA Table	df	F	p
Year	5	1.400	0.230
Weight	1	130.414	0.000
Year* Weight	5	5.305	0.000

Pair-wise Comparison		1977 (n=24)	1982 (n=24)	1986 (n=24)	1992 (n=12)	1996 (n=19)	1999 (n=19)
Slope		0.029	0.027	0.026	0.027	0.012	0.004
	1977		3.120	3.738	3.780	0.080	0.014
	1982			2.535	0.978	0.104	0.014
	1986				1.730	0.077	0.014
	1992					0.099	0.014
	1996						0.966
	1999						

Table 8. p values from Tukey test comparison of inter-year elevations of regression lines from 1977 to 1999 utilizing LSMEANs of ANCOVA for the relationship between fish weight and mirex concentration. General linear model in SPSS version 10.0 (SPSS Inc.).

	1977	1982	1986	1992	1996	1999
1977		0.005	0.000	0.000	0.000	0.000
1982			0.501	0.129	0.002	0.000
1986				0.296	0.008	0.000
1992					0.306	0.000
1996						0.000
1999						

Table 9. Average Mirex Concentrations in Lake Ontario Biota Over Time. Concentrations are in mg/kg wet weight and ND represents no data available.

	1976 (Norstrom *et al.* 1978)	1986 (Flint and Stevens 1989)	1992 (Kiriluk *et al.* 1995)	2000 (Present Study)
Alewife	0.19	0.13	0.034	0.010
Yellow Perch	(TFM 1977) 0.08	0.055	ND	0.001
Zooplankton	ND	0.0035	<0.001	0.0002

Appendix I. Quality Control: Mirex Spike Recovery Efficiency

* 5 gram aliquots of tissue samples were spiked with a known amount of mirex and recovery efficiencies were determined utilizing Soxhlet Extraction, Florisil Cleanup and GC/ECD analysis.

Std. Dev. = Standard Deviation
% RSD = Percent Relative Standard Deviation

Replicate	Actual (mg/kg)	Expected (mg/kg)	Percent Recovery
#1	0.218	0.200	109.2
#2	0.219	0.200	109.5
#3	0.261	0.200	130.7
Mean	0.233		116.5
Std. Dev	0.0246		12.34
%RSD	10.59		10.59

Appendix II. Quality Control: Sample Replication Precision

Results for sample RT-4-00, from 2000 Rainbow Trout collection, analyzed by Soxhlet Extraction, Florisil Cleanup and GC/ECD analysis. Percent Relative Standard Deviations (%RSD) are given. All values are in mg/kg.

Replicate	Mirex	8-monohydromirex
1	0.0159	0.0098
2	0.0234	0.0133
3	0.0214	0.0132
4	0.0229	0.0133
Mean	0.0209	0.0124
Std Dev.	0.0034	0.0017
%RSD	16.32	14.13
Min	0.0159	0.0098
Max	0.0234	0.0133

Appendix III. Quality Control: Comparison of GC/ECD and GCD results

These results are a comparison of average concentrations and ranges of mirex and 8-Photomirex in lake trout tissue from analyses on both GC/ECD and GCD. All results are in mg/kg of analyte.

	GC/ECD		GCD	
	Mirex	8-Photomirex	Mirex	8-Photomirex
Mean	0.182	0.054	0.194	0.056
Std Dev	0.087	0.024	0.108	0.033
SEM	0.033	0.009	0.041	0.012
Range	0.101-0.414	0.030-0.123	0.096-0.358	0.032-0.104
% Difference	6.7%	3.7%		
t-test P value	0.826	0.919		

Appendix IV. Quality Control: Analytical Technique Comparison

These results are statistical comparisons of the mirex concentrations of the same fish (Chinook 1 1992) analyzed by Soxhlet Extraction, Florisil Cleanup and GC/ECD by different analysts to compare analytical technique.

Replicate	Merner 1992	Damaske 2001
1	0.23	0.28
2	0.24	0.26
3	0.27	0.29
4	0.24	
5	0.36	
Mean	0.27	0.28
Std Dev	0.05	0.02
t-test P value	0.799	

These results are statistical comparisons of the mirex concentrations of the same fish (K14A 1982) analyzed by Soxhlet Extraction, Florisil Cleanup and GC/ECD by different analysts to compare analytical technique.

Replicate	Kent 1982	Damaske 2001
1	0.21	0.19
2		0.24
3		0.14
4		0.15
Mean		0.18
Std Dev.		0.05
t-test P value		0.291

Appendix V: Quality Control: Laboratory Comparison

All alewife and salmon samples were analyzed by GC within four weeks of being extracted. The column used to identify mirex and 8-photomirex was an Ultra-2 (12m x .2mm x .33μm) dimethylpolysiloxane capillary column, that was not successful at separating 8-photomirex peaks, as mirex: photomirex ratios (P/M) in salmon were high (average 1.77) compared to the 0.38 average P/M that have been consistent in salmon since 1977. Sample extracts were re-run with a new capillary column (HP-5 fused silica, 30m x 0.25mm x 25-μm i.d.) once the GC was moved to the Lennon Hall laboratory approximately nine months after the samples were extracted. On average there was a ten percent decrease in mirex concentration for the salmon samples that were re-run with the new column nine months after their extractions (Table A). However, a paired t-test indicated that there was no significant difference (P= 0.11, one tail) between the mirex concentrations of the salmon extracts that were run before and after the new column was installed (Table A). However, the average mirex concentration of the 12 salmon according to the Ultra-2 column was 0.116mg/kg, which is above the action limit for mirex. The average mirex concentration of those same 12 salmon according to the HP-5 column, eight months later was 0.088mg/kg, which is below the action limit for mirex. There were also differences in regression line slopes and elevations, however these differences are due to one fish, Ch-2-99 (see graph A). Ch-2-99 was re-extracted and rerun. The original mirex concentrations from the Ultra-2 column were reported in this paper along with the new value for Ch-2-99. Photomirex concentrations from the HP-5 column were reported in this paper.

These differences are not due to an error in making mirex standards. New mirex standards were made after the new HP-5 column was installed. The peak areas of my standards

are similar over time (except when compared to the standards run when the split ratio was 200:1) and similar to the area of standards that Ted prepared (Table B).

The monthly samples of alewives were also run by GC with the two different columns (Ultra-2 and HP-5) nine months apart. Alewife mirex concentrations were also compared between the two different columns. It appears that the mirex in the extracts from the May and June alewives degraded; there was an approximate 75% decrease in mirex concentrations. However, July, August, and September extracts did not appear to break down, concentrations actually increased (Table C). These increases were most likely due to the inaccurate method of reconstituting the sample vials after the solvent had evaporated. A paired t-test indicated that the mirex concentrations of the alewife extract run with the HP-5 column was significantly different ($P=0.02$ one tail) than the same extract run with the Ultra-2 column. The average mirex concentration increased from 0.007mg/kg from the Ultra-2 to 0.009mg/kg from the HP-5 column.

Two alewife samples from May, June and July were re-run yet again to determine if this the difference in the May and June samples was correct. The increasing trend from May to July was the same, however all samples were much lower indicating that photo degradation had occurred.

Three age two alewives from May of 2001 that had not yet been extracted were analyzed with the HP-5 column within a week of their extraction. The mirex concentrations of these fish are in the range (fish concentrations 0.007-0.009mg/kg and vial concentrations of 0.013-0.018mg/kg) of the alewives run with the Ultra-2 column within a month of their extraction confirming the initial results.

The tissue of the four to five alewives that were originally extracted and analyzed for mirex were freeze dried for stable isotope analysis. The only tissue available that was analyzed by both the Ultra-2 and HP-5 columns is from older alewives. Four age-four alewives were re-extracted, re-analyzed and compared to the results from the two different columns. There appears to be an increase in mirex concentration after it was re-extracted and rerun compared to the concentration that was analyzed nine months after the extraction (Table D), however increases are only slight. The old extract was re-run to determine if further breakdown of mirex occurred (indicated by a decrease in mirex concentration and an increase in 8-photomirex) since the initial analysis with the HP-5 column. It does appear that the mirex vial concentrations decreased and the 8-photomirex concentrations increased since they were run last in June.

Three age two alewives from 5/22/00 that had not yet been extracted were extracted to determine if their mirex concentrations are also similar to the concentrations of the original May alewives analyzed by the Ultra-2 column. These concentrations are listed in Table E1 and the results are similar to the original mirex concentrations from the Ultra-2 column, which are listed in Table E2.

Based on this investigation, we decided that photodegradation was the cause of the decrease in the mirex concentrations in the May and June samples of alewives between their analysis in the old laboratory and with the new column in the new laboratory. In this paper, we also reported the old mirex concentrations and the new photomirex concentrations for all the alewives.

Table A. Differences in mirex concentration between the old and new laboratory

Sample	Weight	New Lab	Old Lab	% Diff	
CO-1	5.45	0.123433459	0.088958	-38.7551	
CO-4	4.54	0.138775429	0.118378	-17.231	
CO-7	5.11	0.106448686	0.116757	8.829022	
CH-3	9.54	0.105389989	0.12779	17.52858	
CH-4	7.16	0.028598003	0.024801	-15.3094	
CH-7	11.36	0.085439041	0.102234	16.42811	
CO-2	1.36	0.025878249	0.044891	42.3529	
CO-3	2.61	0.04182258	0.051123	18.19249	
Ch-1	11.82	0.0925031	0.117069	20.98402	
CH-2	8.64	0.254784811	0.504141	49.46164	
CH-5	8.07	0.021497437	0.022333	3.742221	
Average		0.088	0.116	**9.656688**	Avg % Diff

Table B. Mirex standard concentrations and peak areas throughout the study in mg/kg.

Standard Concentration and date	Column Used	Split Ratio	Peak Area
0.01 (8/00BD)	Ultra-2	200:1	240
0.01 (9/00BD)	Ultra-2	50:1	2143
0.01 (12/00) SUPELCO	Ultra-2	50:1	1638
0.01 (6/01BD)	HP-5	50:1	1313
0.01 (10/31/01BD)	HP-5	50:1	1421, 1511
0.01 (11/1/01 TL)	HP-5	50:1	1690
0.1 (8/00BD)	Ultra-2	200:1	2044
0.1 (9/00BD)	Ultra-2	50:1	16667
0.1 (12/00) SUPELCO	Ultra-2	50:1	15317
0.1 (6/01BD)	HP-5	50:1	9974
0.1 (10/31/01BD)	HP-5	50:1	11518
0.1 (11/1/01 TL)	HP-5	50:1	12498

Table C. Differences in mirex concentrations in alewives between old and new laboratories

fish	mirex fish conc (ppm) old lab	Mirex fish conc new lab	Difference	%Diff
Aw-3 (8/14/00)	0.0117	0.002	0.00998	85.21
Aw-4 (8/14/00)	0.0068	0.01	-0.00274	-40.46
Aw-5 (8/14/00)	0.0065	0.009	-0.00291	-44.60
Aw-6 (8/14/00)	0.0045	0.01	-0.00566	-127.00
Aw-7 (8/14/00)	0.0068	0.013	-0.00637	-94.32
Aw-8 (5/22/00)	0.0068	0.002	0.00506	74.51
Aw-9 (5/22/00)	0.0092	0.001	0.0078	84.85
Aw-10 (5/22/00)	0.0061	0.005	0.00097	15.92
Aw-17 (5/22/00)	0.0071	0.003	0.00387	54.63
Aw-1 (9/14/00)	0.0126	0.012	0.00052	4.14
Aw-3 (9/14/00)	0.0104	0.013	-0.00238	-22.90
Aw-4 (9/14/00)	0.0105	0.019	-0.00817	-77.70
Aw-5 (9/14/00)	0.0111	0.01	0.00087	7.80
Aw-7 (9/14/00)	0.0058	0.007	-0.00095	-16.53
Aw-2 (7/19/00)	0.0092	0.011	-0.00144	-15.69
Aw-3 (7/19/00)	0.0083	0.009	-0.00115	-13.85
Aw-4 (7/19/00)	0.0076	0.014	-0.00633	-83.57
Aw-5 (7/19/00)	0.0061	0.013	-0.00663	-109.09
Aw-6 (7/19/00	0.0126	0.014	-0.00149	-11.87
Aw-1 (6/19/00)	0.0073	0.011	-0.00405	-55.19
Aw-6 (6/19/00)	0.0108	0.003	0.00786	72.79
Aw-7 (6/19/00)	0.0021	0.003	-0.00118	-56.11
Aw-8 (6/19/00)	0.0072	0.002	0.00521	72.09
Aw-1 (10/27/00)	0.0042	0.022	-0.01816	-430.66
Aw-4 (9/7/00)	0.0057	0.009	-0.00328	-57.67
Aw-5 (9/7/00)	0.0046	0.007	-0.00217	-46.91
Aw-6 (9/7/00)	0.0077	0.014	-0.00591	-76.26
Aw-11 (9/7/00)	0.007	0.011	-0.00367	-52.16
Aw-8 (9/7/00)	0.0082	0.018	-0.00957	-116.50
Aw-4 (9/29/00)	0.0024	0.002	0.00033	13.86
Aw-6 (9/29/00)	0.0022	0.003	-0.00121	-55.26
Aw-7 (9/29/00)	0.0017	0.01	-0.00826	-481.30
Aw-8 (9/29/00)	0.0022	0.003	-0.0005	-22.70
Aw-3 (9/29/00)	0.0049	0.009	-0.00433	-87.74
Aw-14 (9/29/00)	0.0036	0.004	-0.0003	-8.56
Average	0.007	0.009		

Table D. Mirex and 8-photomirex vial concentrations of Age 4 alewives collected 5/22/00 pesticide concentrations in mg/kg.

Sample	Ultra-2	HP-5		HP-5 (11/5/01)		Re-extracted HP-5	
	Mirex	mirex	8-photo	mirex	8-photo	mirex	8-photo
#1	0.032	0.032	0.012	0.018	0.012	0.048	0.020
#2	0.033	0.042	0.020	0.002	0.040	0.039	0.042
#3	0.045	0.040	0.020	0.037	0.035	0.053	0.054
#4	0.072	0.127	0.088	0.053	0.046	0.067	0.048

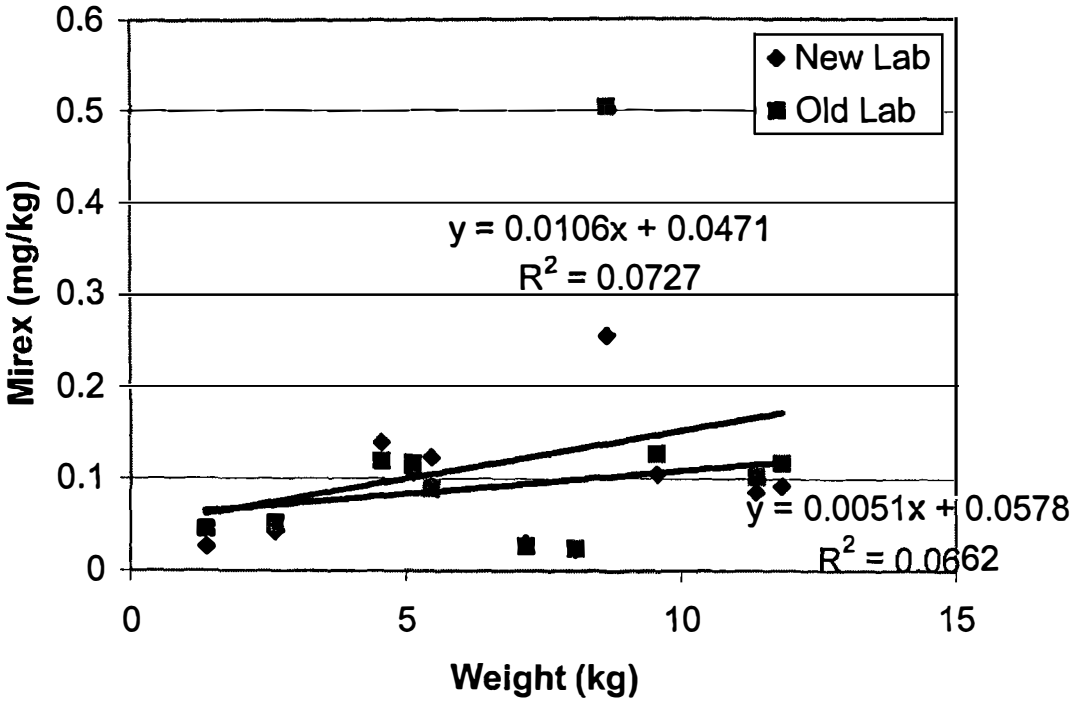
Table E1. Mirex and 8-photomirex vial concentrations in Age 2 Alewives from 5/22/00 that had not yet been analyzed.

Sample	Mirex area	Mirex vial conc	Mirex Fish	Photomirex area	Photomirex vial conc	Photomirex Fish
#11	1585	0.010	0.005	1560	0.012	0.006
#13	3273	0.020	0.010	2707	0.022	0.011
#14	2132	0.013	0.007	2055	0.016	0.008

Table E2. Mirex Vial concentrations and fish concentrations for Age 2 Alewives from 5/22/00 extracted fall of 2000, run by the Ultra-2 column and HP-5 column.

	Ultra-2 Column 200:1 Split 0.19ppm std = 2044 area			HP-5 Column 50:1 Split 0.1ppm std=9974 area		
Sample	Mirex Area	Vial conc	Fish	Mirex Area	Vial Conc	Fish
#8	315	0.014	0.007	397	0.003	0.002
#9	426	0.018	0.009	321	0.003	0.002
#10	705	0.030	0.006	2444	0.026	0.005
#17	325	0.014	0.007	729	0.006	0.003

Mirex concentrations of salmon run in new and old labs



Appendix VI: Two Factor Alewife ANOVA Tables
Factors: Age Class and Month

Table A. Log Transformed Alewife Mirex Concentration

Source	df	F	p
Age	1	22.28	<0.001
Month	4	3.16	0.022
Age*Month	4	2.39	0.064

P values for Tukey Test Results of Alewife mirex concentrations for age 2 and age 4 fish

	May	June	July	Aug	Sept
May		1.000	0.533	0.229	0.009
June			0.712	0.388	0.036
July				0.977	0.421
Aug					0.827

Table B. Log Transformed Alewife Photomirex Concentration

Source	df	F	p
Age	1	4.19	0.046
Month	4	1.25	0.301
Age*Month	4	5.52	0.001

Table C. Square Root Transformed Alewife Weight

Source	df	F	p
Age	1	50.72	<0.001
Month	4	1.03	0.399
Age*Month	4	1.32	0.275

Table D. Log Transformed Alewife Percent Lipid

Source	df	F	p
Age	1	5.86	0.019
Month	4	1.79	0.146
Age*Month	4	0.86	0.498